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Research Article

Diversity of Wild Bees along Elevational Gradient in an Agricultural Area in Central Java, Indonesia

Imam Widhiono, Eming Sudiana, and Darsono Darsono

Faculty of Biology, Jenderal Soedirman University, Jl. Dr. Soeparno no. 63 Purwokerto, Central Java 53122, Indonesia

Correspondence should be addressed to Imam Widhiono; imamwidhiono@yahoo.com

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Increases in mean temperature affect the diversity and abundance of wild bees in agricultural ecosystems. Pollinator community composition is expected to change along an elevational gradient due to differences in the daily ambient temperature. This study investigated the diversity and abundance of wild bees in an agricultural area along an elevational gradient in Central Java, Indonesia. Wild bees were collected using a sweep net in 40 green bean (*Phaseolus vulgaris*) cultivation sampling locations at seven different elevations (8, 108, 224, 424, 644, 893, and 1017 m above sea level). Species diversity was determined using the Shannon–Wiener diversity index. We identified 932 individuals from 8 species of wild bee belonging to 3 families. The family Apidae was predominant, with 6 species, while only 1 species was found from each of Megachilidae and Halictidae. Across the study sites, diversity increased with increasing elevation ((H') = 1.4, (D) = 0.25, and (E) = 0.78 at low elevation to (H') = 2.04, D = 0.13, and E = 0.96 at high elevation), and higher numbers of species were found at middle and high elevations. Species richness and abundance increased linearly with increasing elevation, and species diversity was highest at middle elevations.

1. Introduction

Wild bees (order Hymenoptera: Apoidea) represent the most important group of pollinator insects because they play a key role in agriculture, pollinating almost all crop varieties. However, they are increasingly at risk of local and even global extinction due to climate change [1], which can disrupt the overlap of flower production and pollinator flight activity [2]. The major characteristic of climate change is an increase in the mean global temperature. Elevated temperatures are known to influence the foraging activity, body size at maturity, and individual lifespan [3] of wild bees. The physiological impacts of climate warming might not have negative effects on individual insect pollinators; in fact, some could even have positive effects. Increases in mean temperature affect the diversity and abundance of wild bees in agricultural ecosystems.

Elevational gradients can be used as model systems for climatic change to analyse the role of environmental filtering effects on animal communities [4]. Comparative studies of species ecology along elevational gradients can provide clues to the likely response of both species and communities in the study of the effects of increasing temperature on the diversity and abundance of pollinator insects. However, there are significant differences among the characteristics of typical elevational gradients. Pollinator community composition is expected to change along an elevational gradient due to differences in ambient daily temperature. Due to the harsher environmental conditions, the number of pollinator species tends to decrease with increasing elevation [5]. The relative abundance of Hymenoptera has been negatively correlated with increasing elevation [6]. However, some studies have found that Hymenoptera, especially wild bees, are the most abundant floral visitor at high elevations, at least for some plant species [7]. Total pollinator richness [8] and abundance [9] have been found to decrease with increasing elevation, with plants becoming more ecologically specialised at higher elevations [10]. However, to the best of our knowledge, the wild bee diversity and abundance along an elevational gradient in agricultural areas has not previously been studied

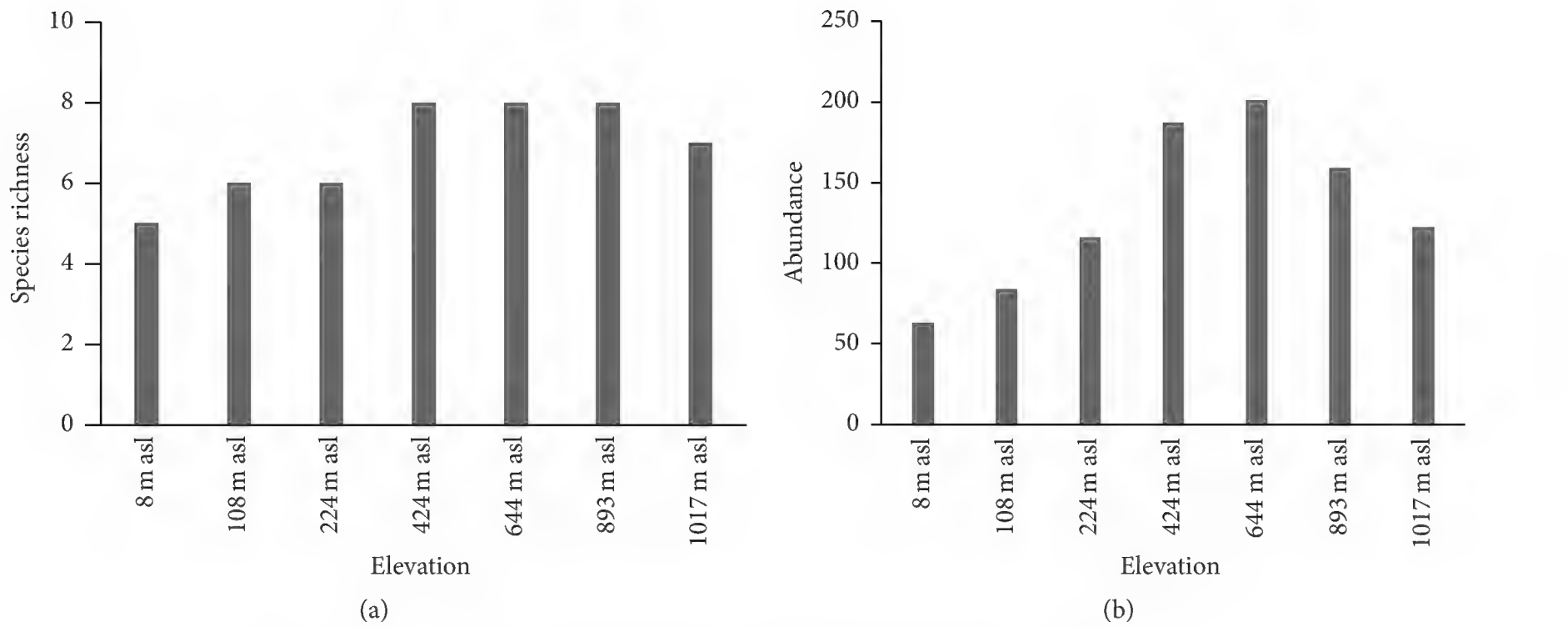

Figure 1: Species richness (a) and abundance (b) of wild bees among elevation.

in Java. In this study, we investigated the diversity and abundance of wild bees in an agricultural area along an elevational gradient to test the hypothesis that elevation is a significant factor underlying the species composition and abundance of wild bees.

2. Materials and Methods

2.1. Study Area. The study was conducted in areas of green bean *(Phaseolus vulgaris)* cultivation at seven locations along an elevational gradient: Lomanis, Cilacap Regency (7°41′06 32″N, 109°01′12 24″E, 8 m above sea level (asl)), Gunung Tugel, Purwokerto (7°28′03 92″N, 109°14′06 35″E, 106 m asl), Banteran, Sikapat, and Banyumas Regency (7°20′00 43″N, 109°16′29 49″N, 224, and 442 m asl, resp.), Limpakuwus and Tuwel, Tegal Regency (7°09′46 38″N, 109°09′06 66″E, 664 and 887 m asl), and Serang, Pubalingga Regency (7°14′48 41″E, 109°17′28 91″N, 1077 m asl). In the regions, green bean is a minor vegetable crop that extensive planting is usually very small and located on land planted with other types of vegetables such as long bean, but, as for research purposes, this plant was chosen because it was found at all altitudes.

2.2. Sampling of Bee Diversity and Identification. The seven long bean plantations were sampled twice each month from April to June, 2014. We collected 100 plant individuals at each site, using the standard sweep net procedures described by Brosi et al. [11] (2008) to sample the bees. During each sampling session, 14 members of the field team (two persons per location) aerially netted bees over 30 min periods. Bees both on flowers and in flight were collected individually with the sweep net. Sampling was performed in the morning (07:00–09:00 local time) on fine days. Bee species were identified by the Indonesian Institute of Science, Bogor. Voucher specimens are held at the Entomology Laboratory, Faculty of Biology, Jenderal Soedirman University, Indonesia.

2.3. Statistical Analyses. The species richness and abundance of wild bees recorded in the seven plots were analysed. A general linear model was applied using SPSS ver. 18.0 software. The dependent variables were wild bee species richness and abundance, and the categorical variables were elevation and daily temperature. Raw data from the field were used to reveal species richness, species diversity (Shannon–Weiner index), component of dominance (Simpson dominance index), and relative abundance of different species at each sampling site [12]. Comparisons of species composition according to location were performed using single linkage cluster analysis based on Bray–Curtis similarity. All diversity parameters were analysed using Biodiversity Pro software [13].

3. Results and Discussion

3.1. Species Composition. In total, 932 individual wild bees belonging to 8 species, 5 genera, and 3 families were collected during the study period. Apidae was the most dominant family, with 6 species *(Xylocopa latipes, X. confusa, X. caerulea, Apis cerana, A. dorsata, and Amegilla cingulata)*, and two families (Megachilidae and Halictidae) were represented by one species each. The composition of wild bee species found at the seven elevations varied, with the largest number of species (8) at the middle-to-high elevations (442–1017 m asl), and the lowest species richness at the lowest elevation (8 m asl), with only 3 species. *Xylocopa latipes, X. confusa, Apis cerana,* and *Megachile relative* were observed at most elevations. *Apis dorsata* and *Xylocopa caerulea* were found only at the middle and high elevations (Figure 1(a)). Species richness differed significantly among elevations (analysis of variance (ANOVA): $F_{6,49} = 5.445$, $p = 0.000223 < 0.05$). Increasing elevation increased wild bee species richness up to 893 m asl ($R^2 = 0{,}84$) and then decreased species richness above 1017 m asl (Table 1).

Table 1: Species composition, abundance, and diversity of wild bees and seven different elevations.

Species	Elevation m above sea level (asl)							Abundance	Relative (%)
	8	108	224	424	644	893	1017		
Xylocopa latipes	18	26	25	27	26	22	16	160	17,16
Xylocopa confusa	15	14	19	44	26	19	23	160	17,16
Xylocopa caerulea	0	0	0	15	29	31	22	97	10,4
Apis cerana	20	23	37	44	38	27	0	189	20,2
Apis dorsata	0	0	0	21	26	16	11	74	7,9
Amegilla cingulata	1	4	11	8	22	13	17	76	8,1
Megachile relativa	9	10	11	16	20	18	19	103	11,05
Lasioglossum malachurum	0	7	13	12	14	13	14	73	7,8
Abundance	63	84	116	187	201	159	122	932	
Relative (%)	6,7	9,01	12,44	20,06	21,56	17,06	13,09		
Mean temperature ˚C	34,3	32,5	31,2	30,4	29,8	29	28,7		
Shannon_H	1,408	1,622	1,684	1,93	2,045	2,035	1,919		
Simpson_1-D	0,740	0,778	0,7944	0,8361	0,8665	0,8634	0,8498		
Evenness_e^H/S	0,817	0,843	0,897	0,860	0,966	0,956	0,973		

3.2. Abundance. In total, 932 individual wild bees belonging to 8 species, 5 genera, and 3 families were recorded. Site 1 supported 4 species with 63 individuals (5.8% of all individuals); Site 2, 5 species and 84 individuals (9.01%); Site 3, 5 species and 116 individuals (12.44%); Site 4, 7 species and 187 individuals (20.06%); Site 5, 8 species and 201 individuals (21.5%); Site 6, 8 species and 169 individuals (17.06%); and Site 7, 7 species and 122 individuals (13.09%) (Figure 1(b)).

Wild bee abundance differed significantly among sites (ANOVA: $F_{6.49} = 3.478$, $p = 0.006 < 0.05$). The most abundant species was *Apis cerana* with 189 individuals (20.2%), followed by *X. confusa* and *X. latipes*, each with 160 individuals (17.16%), *M. relativa* with 103 individuals (11.05%), *X. caerulea* with 97 individuals (10.4%), *A. cingulata* with 76 individuals (8.1%), and *A. dorsata* with 74 individuals (7.9%), and the least abundant was *L. malachurum* with 73 individuals (7.8%). Comparing the wild bee abundance among sampling events at all elevations, the highest abundance was from sampling number 2 with 199 individuals (21.3%), followed by sampling number 1 with 172 individuals (18.4%), sampling number 4 with 157 individuals (16.8%), sampling number 3 with 148 individuals (15.8%), sampling number 5 with 136 individuals (14.5%), and the lowest abundance was from sampling number 6 with 120 individuals (12.8%). However, statistical analysis revealed no significant differences among sampling events (ANOVA: $F_{5.42} = 1.01800$ $p = 0.419 > 0.05$).

Across the study sites, diversity increased with increasing elevation (Shannon–Wiener index (H') = 1.4, dominance (D) = 0.25, and evenness (E) = 0.78 at low elevation to (H') = 2.04, D = 0.13, and E = 0.96 at high elevation), and higher numbers of species were found at middle and high elevations. The distribution of wild bees among the sites was significantly influenced by elevation. The richness, abundance, diversity, and evenness of wild bees at different sites are presented in Table 1.

Analysis of community structure similarity among elevations showed that the three sites categorised as low elevation (8–224 m asl) had high similarity (84.53%), the two middle-elevation sites (424–644 m asl) had 83.50% similarity, and the two high-elevation sites (893 m–1017 m asl) had 79.7% similarity. However, the highest species similarity (87.2%) was found between Site 5 (644 m asl) and site 6 (893 m asl) (Figure 2).

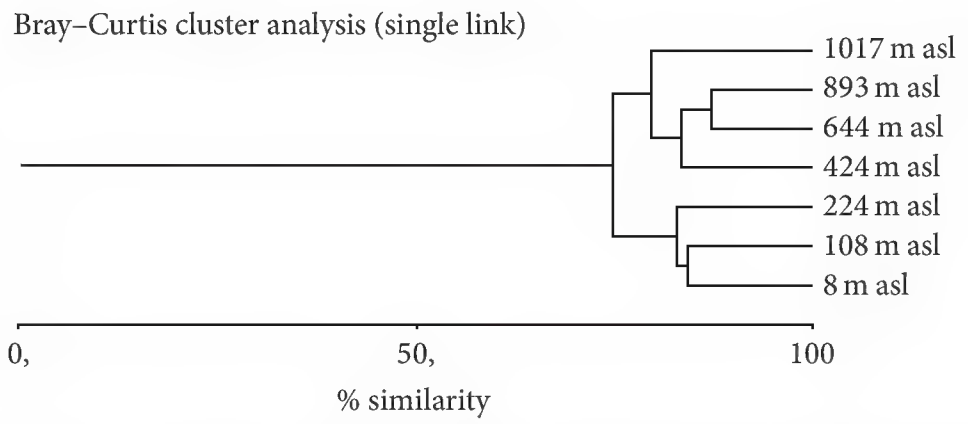

Figure 2: Similarity (Bray–Curtis) single linkage of wild bee in seven different elevations.

4. Discussion

The number of wild bee species and the species composition recorded in this study were similar to those reported in previous studies in various regions. Hoiss et al. [14] observed 10 species of wild bees at *Vigna unguiculata* in Ghana belonging to the families Apidae (*Xylocopa, Apis*, and *Amegilla*), Megachilidae (*Megachile eurymera*), and Halictidae (*Nomia* sp.). Kwapong et al. [15] reported that the main insects observed on cowpea flowers were bees such as *Apis mellifera, Ceratina* sp., *Megachile* sp., *Xylocopa calens, Xylocopa imitator, Braussepis* sp., *Lipotriches* sp., *Melecta* sp., and *Amegilla* sp. Kingha et al. [16] reported *Apis mellifera* as the most abundant insect species visiting *P. vulgaris* flowers in Kenya, followed by *Xylocopa calens* and *X. inconstans*. The flowers of long bean attract wild bee pollinators from Apidae, probably because the members of this family have sufficient strength to open the flower to collect pollen and nectar [17]. However, to the best of our knowledge, to date there have been no studies on insect pollinator diversity in green bean cultivation areas

in Indonesia for comparison purposes. In Sukabumi, West Java, in mustard *(Brasica rapus)* at an elevation of approximately 854 m asl, Aouar-Sadli et al. [18] observed insect pollinators from the order Hymenoptera, family Apidae, subfamily Apinae, consisting of the species *Apis cerana, Apis dorsata,* and Trigona sp., from the subfamily Xylocopinae, the species *Xylocopa caerulea, X. confusa, X. latipes,* and Ceratina sp., from the family Colletidae, subfamily Hylaeinae *Hylaeus sp.*, from the family Halictidae, subfamily Nomiinae *Nomia* sp., and from the family Scoliidae *Compsomeris lindenni.* Our study findings indicate that the species composition of insect pollinators on agricultural land is dominated by wild bees.

Elevation strongly influenced the diversity and abundance of wild bee communities. Species richness and abundance increased linearly with increasing elevation, and species diversity was highest at middle elevations. These results are inconsistent with those of Gottlieb et al., [19], who reported that the diversity and abundance of wild bees decreased linearly with increasing elevation from 60 to 2000 m asl. This difference may be due to differences in the geographical region, climate zone, and range of elevational gradient. A commonly reported pattern in species richness along elevational gradients is a unimodal distribution with a mid-elevation peak [20]. In our study, peak species diversity occurred at middle elevations due to the presence of two species (*Apis dorsata* and *Xylocopa caerulea*) that began to appear above 442 m asl. The presence of the giant honey bee *(A. dorsata)* at mid-to-high elevations may be due to habitat suitability rather than elevation effects because these bees require a forested habitat with high trees, whereas blue carpenter bees tend to be abundant in cool environments. Large carpenter bees (genus *Xylocopa*) are wood-nesting generalist pollinators of broad geographical distribution. Their foraging is characterised by a wide range of food plants, long season of activity, tolerance of high temperatures, and activity under low illumination levels [21]. Carpenter bees tolerate high ambient temperatures during foraging, and most species are inactive at low temperatures, and these bees were found in all of our study sites. All of the sites from the lowest to highest elevation had daily temperature ranges appropriate for wild bees. Generally, the genus *Xylocopa* is able to move around well in a daily temperature range of 25–35°C [22]. This heat tolerance suggests efficient heat regulation ability in carpenter bees, possibly controlled by a thermoregulatory centre in the prothorax [23]. In contrast, *Apis cerana* has a tendency to forage in conditions of warm temperatures [24]. The presence of the three species *Megachile relativa, Amegilla cingulata,* and *Lasioglossum malachurum* almost evenly at all elevations studied indicates that these species are able to move around in the range of temperatures that occur at these elevations.

5. Conclusion

Eight species of wild bees from four families of the order Hymenoptera were recorded in green bean cultivation areas at seven different elevations in Central Java, Indonesia. Species richness and abundance increased linearly with increasing elevation, and species diversity was highest at middle elevations.

Disclosure

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/A8aul1.

Competing Interests

The authors declare that they have no competing interests.

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Research Article

Sigma Virus (DMelSV) Incidence in Lines of *Drosophila melanogaster* Selected for Survival following Infection with *Bacillus cereus*

Meghan L. Bentz,[1] Eve A. Humphrey,[1,2] Lawrence G. Harshman,[3] and Marta L. Wayne[1]

[1]*Department of Biology, University of Florida, Gainesville, FL 32611, USA*
[2]*Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA*
[3]*School of Biological Sciences, University of Nebraska Lincoln, Lincoln, NE 68588, USA*

Correspondence should be addressed to Marta L. Wayne; mlwayne@ufl.edu

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The immune response of *Drosophila melanogaster* is complex and involves both specific and general responses to parasites. In this study we tested for cross-immunity for bacteria and viruses by scoring the incidence of infection with the vertically transmitted Sigma virus (DMelSV) in the progeny of a cross between females transmitting DMelSV at high frequencies and males from lines subjected to three selection regimes related to resistance to *Bacillus cereus*. There was no significant difference in transmission of DMelSV among selection regimes, though results suggest that the *B. cereus* selected lines had lower rates of infection by DMelSV. We found a significant difference in viral infection with respect to the sex of the progeny, with males consistently less likely to be infected than females. Given a finite energy budget, flies that have experienced immune system challenge may show alterations in other life history traits. Later eclosing progeny were also less likely to be infected than earlier eclosing progeny, indicating a relationship with development time. Finally, there was a significant interaction between the timing of collection and the sex of the progeny, such that later eclosing males were the most resistant group. Increased development time is sometimes associated with increased energy acquisition; from this perspective, increased development time may be associated with acquiring sufficient resources for effective resistance.

1. Introduction

The goal of this study was to test for cross-immunity between bacteria and viruses by scoring the incidence of the vertically transmitted rhabdovirus Sigma (DMelSV) infection in progeny of DMelSV-infected females and males from lines subjected to three selection regimes related to resistance to the Gram-positive bacterium, *Bacillus cereus*. Cross-immunity among viruses has been previously demonstrated for flies selected for survival following infection with DCV [1]. Similarly, if there are overlapping pathways involved in resistance to bacteria and viruses in Drosophila, then selection for resistance to the bacterium might also confer resistance to DMelSV.

The Gram-positive bacterium *Bacillus cereus* might be expected to mount an immune response via the Toll pathway; however, the DAP-type peptidoglycans of the *Bacillus* genus elicit IMD response [2]. There is conflicting evidence with respect to a role for the Toll and IMD pathways in response to infection by DMelSV. One study found that the major resistance gene to DMelSV, *ref(2)P*, was a component of the Toll signaling pathway [3]. In another study, expression of various downstream genes regulated by the Toll pathway was significantly increased in response to DMelSV, and key components of that pathway such as Toll, as well as Relish from the IMD pathway, were upregulated as well; however, neither the Relish nor Toll increases were statistically significant [4]. Yet another study failed to find any evidence of upregulation of either Toll or IMD upon infection with DMelSV [5]. Therefore, we hypothesized that there might be cross-immunity between *B. cereus* and DMelSV infection via either Toll, IMD, or yet another pathway.

Bacillus cereus are an aerobic spore forming Gram-positive bacteria that are closely related to *Bacillus anthracis*. *B. cereus* is commonly found in soil, on vegetable matter, and in human foods (both raw and after cooking). *B. cereus* is a saprophyte in soil and can be an opportunistic pathogen of soil invertebrates. This bacterium can cause medical problems in humans through consumption of bacterial contaminated food, causing several types of self-limiting gastric problems.

DMelSV is a rhabdovirus commonly found in *Drosophila* [6]. DMelSV is transmitted to progeny by a parent of either sex (vertical transmission), but transmission by females is typically more effective [3, 4]. The virus is virulent; DMelSV can result in a reduction in fitness in the laboratory and in field populations [4, 5]. Infection of *Drosophila* by DMelSV results in a characteristic sensitivity to CO_2 [7], such that infected flies will become paralyzed and die when exposed to concentrated CO_2. This sensitivity can be used to infer the presence of the virus in individual flies and thus estimate the prevalence of the virus in fly populations.

We used lines previously selected for resistance to the bacterium *B. cereus* [6, 8] to test the hypothesis that there would be cross-immunity between bacteria and viruses, in this case, DMelSV. As a partial control in the previous selection experiment, some lines were also evolved in response to sterile wounding, which could involve inflammatory processes or other responses connected to the immune system [7, 9, 10], and perhaps similarly result in diminished infection by DMelSV. The test for *D. melanogaster* refractoriness to infection by DMelSV was novel in that the consequences of two regimes of genetic selection on the host flies were investigated in terms of the likelihood of virus transmission. We performed crosses between males from the S, CI, and CN selected lines and females from an unrelated line infected with DMelSV to test for relationships between transmission and selection regime, progeny sex, and development time in these *D. melanogaster* populations that were related by common ancestry but differentiated by selection.

We did not find evidence for cross-immunity; that is, the *B. cereus* selected lines did not have significantly lower rates of infection by DMelSV. There was, surprisingly, a significant difference in viral infection with respect to the sex of the progeny: male progeny were consistently less likely to acquire the virus than were female progeny. Later eclosing progeny were also characterized by higher rates of uninfected flies than earlier ones. Finally, there was an interaction between timing of collection and progeny sex such that late eclosing males were the least likely to be infected. Thus, longer development time appears to be associated with reduced virus acquisition.

2. Materials and Methods

The present study involved progeny from crosses between a stock of female *D. melanogaster* that carried DMelSV with male flies from lines that were selected for survival after infection by *B. cereus* and control lines. The incidence of DMelSV in progeny was assessed by exposing female and male progeny to concentrated CO_2.

2.1. Fly Stock and Lines. Because DMelSV transmission by females is higher than that from males [6], we used infected females in our experiment to explore interactions between resistance to *B. cereus* and transmission of DMelSV. The females used for crosses in our experiment were DMelSV-infected via injection and were effectively isogenic (stock 27, described in detail by Rittschof et al. [11]; this stock is not infected with *Wolbachia*). Flies were cultured under standard light and temperature conditions (12 : 12, light : dark; 25°C) on standard molasses-agar food. Individual vials were set up with a constant density of five females and five males and allowed to lay eggs for five days, for at least three generations prior to the experimental crosses. The experimental crosses were kept under similar light and temperature conditions in standard food vials (described by Rittschof et al. [11]). Flies infected with DMelSV die after concentrated CO_2 exposure [9], which serves as an inexpensive, quick, and reliable way to test for infection. Females were transmitting virus at 100% frequency (i.e., half their progeny were exposed to CO_2 and all of those progeny died) at the start of the study.

Selection was conducted on three replicate lines for each of the three treatments: S (resistance to *B. cereus* infected), CI (response to wounding), and CN (the unperturbed control) for 19 generations prior to the experiments reported here (i.e., selection was relaxed for one generation prior to shipment of flies to UF) [12]. None of the lines were infected with *Wolbachia*, no doubt reflecting the low frequency of *Wolbachia*-infected flies in the original population from which the lines were derived. Following selection, a 3.3-log increase in the number of spores required for 50% mortality was observed [12].

2.2. Experimental Protocol. Nonvirgin, uninfected males from S, CI, and CN lines were crossed to virgin, DMelSV-infected females. Females were held for 12–36 hours prior to crossing. Virginity was verified by checking female holding vials for progeny (larvae or pupae) a week later. No incidents of nonvirginity were discovered. Crosses were performed with a controlled density of five females and five males in each vial. Females were permitted to lay eggs for five days at the standard light and temperature conditions described previously. Females were then assayed for infection via a CO_2 sensitivity assay per the protocol of Wayne et al. [13]; no females recovered from the assay, confirming their positive DMelSV infection status. Nine replicate vials of crosses were used for the CI lines, nine replicated crosses were used for the CN lines, and eight replicate crosses were conducted for the S lines.

Two collections of offspring from the experimental crosses were made: the first was 11 days after the crosses were initiated and the second was 13 days after the crosses were initiated. Thus, day of collection was a proxy for development time. Following collection, progeny were held for 24 hours and then assayed for sensitivity to CO_2 [13].

2.3. Statistical Analysis. The dependent variable was the percentage of flies that did not show CO_2 sensitivity, that is, the percentage of uninfected flies. The percent data was transformed by arcsine square root to improve normality

Table 1: Percent of uninfected progeny by sex and selective regime. Selective regimes included the following: S (resistance to *B. cereus* infected), CI (response to wounding), and CN (the unperturbed control). Percent was calculated as the total number of uninfected progeny divided by total number of progeny.

	S	CI	CN
Males	50/341 = 14.7%	17/232 = 7.33%	26/343 = 7.58%
Females	16/366 = 4.37%	5/268 = 1.87%	6/396 = 1.52%

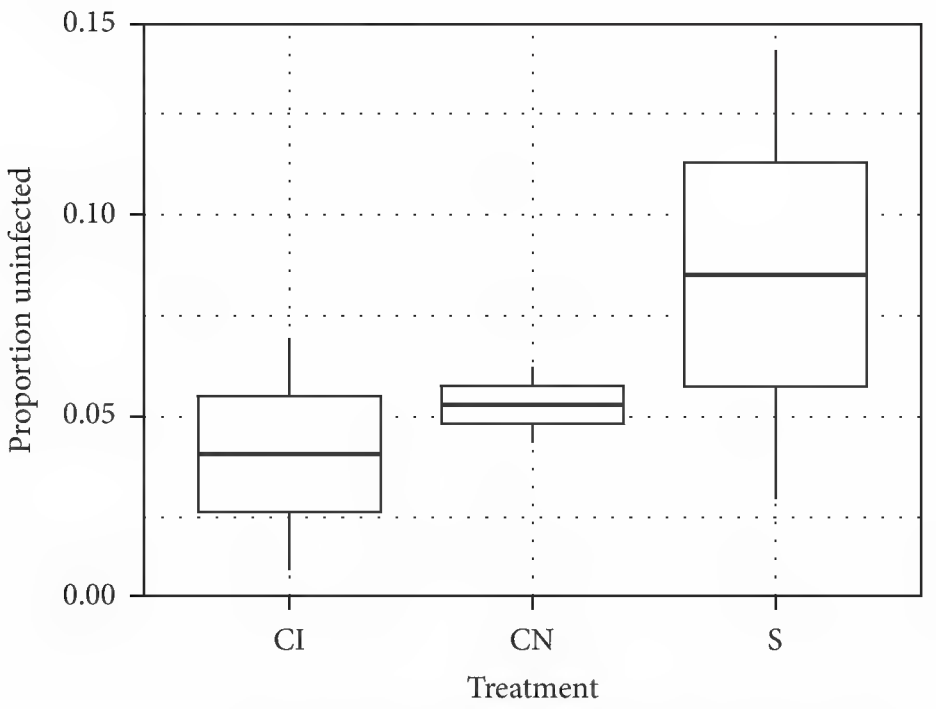

Figure 1: Box plot showing median and interquartile range of uninfected progeny (untransformed data) for each selection regime. Selective regimes included the following: CI (response to wounding), CN (the unperturbed control), and S (resistance to *B. cereus* infected). The median proportion of uninfected progeny was highest for the lines selected for resistance to *Bacillus cereus*.

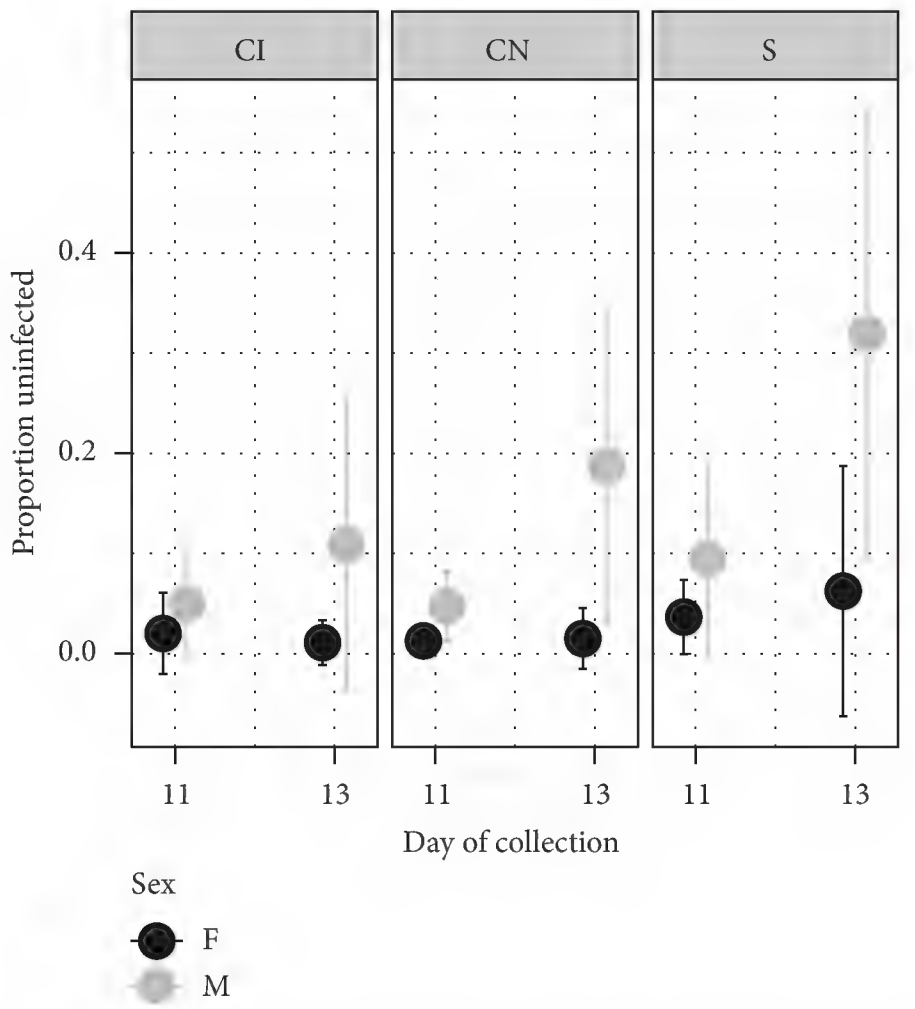

Figure 2: Graph (means ± 2 standard errors) of proportion uninfected progeny (untransformed data) for each selection regime × day × sex combination. Selective regimes included the following: CI (response to wounding), CN (the unperturbed control), and S (resistance to *B. cereus* infected). The significant interaction of day × sex is obvious, with male progeny having higher proportion surviving on the second day of eclosion compared to females across treatments. Again, the lines selected for resistance to *B. cereus* tended to have the highest proportion of uninfected animals, and this trend was notably driven by the male progeny eclosing on the second day of collection.

of the residuals. The data were then analyzed in R using lme4: ANOVA with type III Wald F tests and Kenward-Roger degrees of freedom. The model included the main effects of selection regime, day of collection, and sex of the progeny and main effect interactions.

3. Results

The vast majority (1,826/1,946; >93%) of progeny flies succumbed to CO_2 anesthesia, consistent with infection by DMelSV (Table 1). The number of uninfected male and female progeny from each category of cross between the stock carrying DMelSV and the three types of lines (S = *B. cereus* selected, CI = control wounded each generation, and CN = no perturbation controls) is reported in Table 1. To test for refractoriness to DMelSV infection, we analyzed the proportion of females or males that survived the CO_2 assay (i.e., which were not infected) for each type of cross (Table 2). The main effect of selection regime was not significant (P = 0.518; Table 2; Figure 1) nor were any of its interactions (Table 2). Although a trend in the data (Figure 1) suggests that there were a higher proportion of uninfected animals in the lines selected for resistance to *B. cereus* than in either of the two control treatments, in no case did survival differences between crosses types attain statistical significance.

Males were significantly more likely than females to be uninfected regardless of selection or control regime (Tables 1 and 2 and Figure 2; $P < 2.7\times10^{-4}$). Although transmission by males is generally lower than that by females and mutations blocking male but not female transmission are well known [6], we are unaware of other work demonstrating differences in sex-specific refractoriness to acquisition of DMelSV.

There was a significant effect of development time, as assayed by the day of collection, such that a higher proportion of uninfected flies eclosed by day 13 than that on day 11 (day: $P < 0.027$, Table 2; Figure 2). Moreover, there was a significant interaction between collection date and progeny sex (day × sex: $P < 0.016$, Table 2; Figure 2), such that surviving males were more likely to eclose on the second day of collection than surviving females.

4. Discussion

The present study tested for cross-immunity via differential survival following CO_2 exposure, which is fatal to flies infected with DMelSV, among progeny sired by males of lines

Table 2: ANOVA of transformed proportion of uninfected progeny. The model included the main effects of selection regime, day of collection, and sex of the progeny and main effect interactions. The proportion was transformed by arcsine square root to improve normality of the residuals.

Source	F	D.f.	D.f. resid.	P
Intercept	21.62	1	2	0.043*
Selection regime	1.36	2	1	0.518
Day	5.83	1	18	0.027*
Sex	20.33	1	18	2.7×10^{-4}***
Selection regime × day	1.62	2	18	0.225
Selection regime × sex	1.05	2	18	0.369
Day × sex	7.13	1	18	0.016*
Selection regime × day × sex	1.00	2	18	0.387

*$P < 0.05$; ***$P < 0.001$.

selected for *B. cereus* resistance and progeny sired by control males, both crossed to females from a stock that transmits DMelSV at effectively 100%. Neither selection regime (i.e., selected versus controls) nor any of its interactions were significant, and so we have no evidence for cross-immunity between *B. cereus* and DMelSV, despite the fact that both parasites could involve the Toll and IMD pathways. Failure to detect a significant effect may be the artifact of the small sample sizes in this experiment, small effect size, or both. Given that the trend in the data is consistent with increased survival following CO_2 exposure in the offspring of males selected for *B. cereus* resistance, it is possible that such cross-immunity between bacteria and viruses does exist and that a greater sample size might provide sufficient power to detect a difference in cross-immunity between selected and control lines. However, the current data do not support such a conclusion.

To the best of our knowledge, a sex bias in DMelSV transmission has not been previously reported. The absolute numbers of male and female S progeny were very similar (341 males, 366 females), so the difference cannot be explained by sample size. One possibility is that the virus we used [11] is peculiar such that it mediates sex bias in infected progeny. However, this outcome has not been observed in other studies using the same virus and the same stock 27 ([11]; other unpublished data).

Infection by DMelSV is known to increase development time [14] in infected *D. melanogaster*, and viral titer is positively associated with development time in infected flies [15]. Thus, we were initially surprised that uninfected animals had increased development times relative to infected flies. However, the data documenting increased development time as a result of DMelSV are either from comparisons of infected and uninfected flies [14] or among flies that are all infected but with variable viral titers [15]. Thus, to the best of our knowledge, the relationship between transmission success versus failure and development time among the progeny of infected flies has not been explored.

The cost of mounting an immune response is relevant to life history trait correlations and evolution in animals. It is generally thought that mounting an immune response is physiologically costly and that standing immunity negatively impacts other fitness traits. Populations of *D. melanogaster* have been used for artificial selection for resistance to infection by microbes or parasitoids, and the evolution of other fitness-related traits is a typical result of such studies (summarized by McKean and Lazzaro [16]). Conversely, artificial selection on life history traits can affect immunity; Modak et al. [17] documented that *D. melanogaster* selected for decreased development time exhibited a shorter time to death following introduction of *E. coli* than unselected controls. In the present study, lines of *D. melanogaster* selected for survival after introduction of *B. cereus* tended to have decreased incidence of progeny that acquired DMelSV. Additionally, selection for survival after exposure to *B. cereus* resulted in constitutively slower development time [12], consistent with a tradeoff between immunity and development time. In the data presented here, the proportion of uninfected progeny, that is, progeny with potentially greater immune investment, increased with time (significant day of collection term), again consistent with a tradeoff.

Transmission of DMelSV to progeny was significantly higher in males in all of the lines used for crosses (Table 2, Figure 2). Progeny male refractoriness to acquisition of virus was particularly notable as an interaction between selected lines and day of collection (Table 2, Figure 2), driven by the relatively high frequency of uninfected males on the second collection day. Although this pattern is seen in all selection treatments, it is most pronounced in the S lines (the lines selected for resistance to *B. cereus*; Figure 2).

Vincent and Sharp [18] demonstrated greater resistance (as well as tolerance) to *P. aeruginosa* in male *D. melanogaster* relative to females. They note that higher male resistance is not uncommon in response to actual parasite challenge (realized immunity), while higher female immunocompetence is often observed in uninduced animals (constitutive immunity). In our experiment, offspring were challenged by DMelSV during development, thus representing realized rather than constitutive immunity. Thus, our observation of greater resistance in males (and of increased development time in resistant animals in general and males in particular) is possibly the result of greater investment in immunity and is consistent with the patterns described by Vincent and Sharp [18].

There are two general mechanisms, which are not mutually exclusive, that could underlie an association between delayed development time and refractoriness to DMelSV transmission. The first is that delayed development results in an increase in time available for the accumulation of food resources, which could then be used to mount a successful immune response and which in our study could result in resistance to DMelSV. A second possibility is that delayed development time is associated with a molecular process that directly results in increased immunity. As one possibility, ecdysone signaling is an innate immunity maturation or immunity-stimulating hormonal agent [19, 20]. Further investigation of the relationship between host development time and successful transmission of DMelSV will help distinguish between these hypotheses.

5. Conclusions

There was no statistically significant impact of fly resistance to *B. cereus* on virus transmission incidence. However, there was a weak association between resistance to *B. cereus* and diminished transmission of DMelSV. Moreover, there were statistically significant effects of progeny sex, timing of collection, and their interaction: males eclosing later were associated with reduced virus transmission. The selected lines were previously found to develop more slowly, and, in this experiment, animals from the second collection (i.e., slow developers) were more likely to be resistant. If longer development time is generally associated with reduced virus transmission, it would be interesting to investigate the developmental maturation of the innate immune system in *D. melanogaster* and the interaction between nutritional status and maturation of innate immunity. The results of the present study could lead to novel insight into the ontogeny of immunity in this model for genetic and innate immunity research.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Buzzing Wild Bee Visits Enhance Seed Set in Eggplant, *Solanum melongena*

U. J. M. Shanika R. Jayasinghe, T. H. Saumya E. Silva, and W. A. Inoka P. Karunaratne

Department of Zoology, Faculty of Science, University of Peradeniya, 20400 Peradeniya, Sri Lanka

Correspondence should be addressed to W. A. Inoka P. Karunaratne; inokap@pdn.ac.lk

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Sixty percent of the angiosperms with poricidal anthers are buzz-pollinated by bees (Hymenoptera: Apoidea: Apiformes). Plant taxa with *Solanum*-type flowers have larger anthers and shorter filaments. *Solanum melongena* (Solanaceae) is more commonly and efficiently pollinated by buzz pollinators. The present study documented bees and their diurnal pattern of visitation to flowers, relationship between their handling time and flower age, and the effect of bee visits on fruit and seed set in *S. melongena* in two sites in Kandy District. Efficiency of buzz pollination over pollination in the absence of bees was determined using open buds and buds covered with pollinator exclusion bags. On average, 150 days were taken to complete the life cycle of *Solanum melongena*. Three buzzing bees and two nonbuzzing bees in site I and five buzzing bees and two nonbuzzing bees in site II were recorded. Handling time of *Pachynomia* sp. and *Hoplonomia westwoodi* indicates that bees spend more time at new flowers than at old flowers. Handling time is higher in the smaller bee, *Pachynomia* sp., than in the larger bee, *H. westwoodi*. Statistical data on pollinator exclusion experiment revealed that the fruit set and seed set of *S. melongena* are enhanced by buzz-pollinating bees.

1. Introduction

Pollination is one of the most important plant-animal interactions for reproduction of plants [1]. Pollinator organisms are engrossed to pollinate 35% of the plants cultivated in the world [2]. Among them, more than 1500 plant species are pollinated by bees [3]. Crops such as coffee (*Coffea* spp.) [4, 5], melon *(Citrullus lanatus)* [6], tomato *(Solanum lycopersicum)* [7], sunflower *(Helianthus annuus)* [8], canola (*Brassica* spp.) [9], and blueberries (*Vaccinium* spp.) [10] are common examples of bee-pollinated crops. Among pollinator bees, certain clades such as carpenter bees (Xylocopini), bumble bees (Bombini), and orchid bees (Euglossini, Centridini, and Augochlorini) are able to vibrate flowers to collect pollen. Since this strong rapid vibrations made a buzzing sound, this phenomenon is known as "buzz pollination" [11].

Buzz pollination can be seen in 357 genera of plants representing 54 families of angiosperms [12]. Those plants have poricidal anthers, which mean anthers do not split open to release pollen but need natural or artificial vibrations to release pollen through their apical pores [13]. As pollen is well hidden and available only to specialized buzzing bees that vibrate the anthers of flowers, pollen waste is very low in buzz-pollinated flowers [12]. Most buzz-pollinated flowers have few stamens [14] and downward facing position which is positive discrimination to hanging of bees while they buzz [15]. The strong yellow colour of the anthers contrasting markedly with the usual purple or white corolla is a characteristic of buzz-pollinated flowers. Flowers with such strongly contrasting colours within the visible spectrum of light have been shown to be attractive to pollen-gathering bees even in the absence of reinforcing UV patterns [11].

Solanaceae is one of examples of plant families that are pollinated by buzzing bees. Though *Solanum* flowers lack nectar, they afford a moderately wealthy pollen load for bees that visit them [16]. *Solanum* is the second largest genus of flowering plants which contains more than 2000 species [17]. It includes major economical crop species such as *Solanum melongena* (eggplant) and *S. tuberosum* (potato). The eggplant, *S. melongena* (Solanaceae), is a major vegetable

crop in many countries of the world [18]. It is a delicate, perennial, annual tropical plant native to Africa and Asia [19]. Fruits are formed due to both cross pollination [12] and self-pollination [17, 20]. However, according to Buchmann and Cane [17], *S. melongena* is more commonly and efficiently pollinated by buzz pollinators. Several studies had been carried out to test the effect of self-pollination on seed set in *S. melongena* [21–24] in temperate countries of the world. However, very few studies [25, 26] have been conducted in tropical countries on the pollination ecology of *S. melongena*. The present study was designed to fill this vacuum, with the aim to (i) identify bees visiting flowers of *S. melongena* and their diurnal pattern of activity, (ii) record the handling time of buzzing bees at flowers with respect to age of flowers, and (iii) determine the effect of bee visits to flowers on fruit set and seed set.

2. Materials and Methods

Regarding study sites, field work was conducted in Sri Lanka at two sites (Miwatura and Gelioya) in Kandy District (7°15′N, 80°45′E), central Sri Lanka, for a period of 10 months (from June 2008 to April 2009). The mean annual temperature and rainfall of the two sites were 41°C and 700 mm (source: Natural Resources Management Center, Peradeniya), respectively. The site at Miwatura is a semiagriculture research field of University of Peradeniya, located in the western valley of the Mahaweli River, in close proximity to a Peradeniya, Gampola main road. In this site, several vegetable crops belonging to Solanaceae and Leguminosae had been cultivated from time to time for research purposes. The site at Gelioya is surrounded by a paddy field, a home garden, and plots of vegetable crops mainly belonging to Solanaceae, Cucurbitaceae, and Leguminosae.

2.1. Field Preparation and Planting of S. melongena Seedlings. From June 2008 to September 2008, field was prepared and planted at Miwatura site. First, seeds of *S. melongena* were planted in seed beds following agricultural recommendations. Then, 20-day-old germinated seedlings were cultivated in the field for field experiments. Depending on the availability of land, an area of 12 m × 15 m in Miwatura site was selected and 50 *S. melongena* plants were cultivated. At Gelioya site, 80 *S. melongena* plants were present in a 15 m × 15 m area.

2.2. Recording of the Times of Stigma Receptivity and Anther Dehiscence. Times of stigma receptivity and anther dehiscence were observed in freshly opened five flowers of *S. melongena*. The time of stigma receptivity was determined by observing the stigma through a hand lens every 10 minutes from 7.00 a.m. to 3.30 p.m. The stigma was touched by a needle tip to observe the stickiness and was considered as the time of stigma receptivity. The time of anther dehiscence was observed by shaking the flower onto a white paper every 10 minutes from 7.00 a.m. The time at which pollen was released and collected onto the white paper was considered as the time of anther dehiscence.

2.3. Study of Bee Visitors, Species Composition, and Their Diurnal Activity. Over a period of 5 months (September 2008 to January 2009), bees visiting *S. melongena* flowers were observed at the two sites. Bees visiting flowers of *S. melongena* were observed for ten sunny days until no new species were recorded. Bees that visited flowers were categorized into two groups as buzzing or nonbuzzing bees. Buzzing bees were identified by carefully listening to the sound they produce at the time of pollen collection. Bees that collect pollen from anthers without producing a sound were considered nonbuzzing bees. Bees collected using sweep net were curated and identified using published keys [27]. Reference specimens were logged in the Invertebrate Systematics and Diversity Facility (ISDF) of the Department of Zoology, University of Peradeniya.

The time of visits of each species was observed from 07:00 to 15:30 at 30-minute intervals on two sunny days at the Miwatura site and four sunny days at the Gelioya site. However, no attempt was made to count the number of individuals (abundance) of different species visiting flowers.

2.4. Determining the Number of Pollen Grains in Anthers of Flowers of Different Age. Pollen from 20 flowers at different age (five flowers from each stage) was counted to determine the relationship between buzzing time and available pollen grains at Gelioya site. Flowers at four different ages, newly opened flowers, one-day-old flowers, two-day-old flowers, and flowers that were more than two days old, were selected. Newly opened flowers were observed on 27 September 2008. On the following day, these flowers were considered to be 1 day old and, on the third day, these flowers were considered to be 2 days old. On the fourth day, these 20 flowers were considered to be >2 days old. From each flower, one anther was removed and cut open longitudinally to collect all the grains. From each anther, the pollen grains were put into 1.5 ml of 50% alcohol in separate watch glasses. From each sample, 1 ml of the mixture (after stirring) was transferred into McMaster Chamber. The average number of pollen grains in each chamber was counted. The available pollen grains per 1 ml of the four mixtures were calculated.

2.5. The Relationships between the Handling Time of Bees at Flowers and Age of Flowers. Handling time (total time that a particular bee buzzes at a flower during a single visit) was recorded for two of the common buzzing bee species (*Hoplonomia westwoodi* and *Pachynomia* sp.) at the Gelioya site. Flowers of different ages, newly opened, one-day-old, two-day-old, and more than two-day-old flowers, were selected by following the above-mentioned way. Flowers of different age groups were tagged using different coloured tags. Five flowers were selected from each age category and the handling time (in seconds) in each of the selected flowers by the two common bee species was recorded. The procedure was followed on two sunny days for *H. westwoodi* (25 December 2008 and 26 December 2008) and *Pachynomia* sp. (10 January 2009 and 23 January 2009). Ten specimens of each of the two common female (male bees do not buzz at flowers to collect pollen) buzzing bee species were measured for body length to investigate the difference between the

body lengths of the two buzzing bee species. The relationship between the handling time and the mean body size (body length from the anterior end of the head to the posterior end of the tip of abdomen) of two more common bee species was also determined.

2.6. Determining the Effect of Bee Visits to Flowers on Fruit Set and Seed Set. Field trials were conducted (from December 2008 to April 2009) at the Gelioya site to determine the efficiency of buzz pollination over pollination in the absence of bees on fruit set and seed set. A sample of 50 bunches of flower buds of *S. melongena* were selected for the study. Of the selected 50 bunches, 25 bunches were tagged and left open for bees to visit. Numbers of fruits formed by each bunch were recorded. Their length (from proximal end to the distal end) and weight of fruits were measured. Fruits were hold until they were rotten and the numbers of seeds produced by each fruit were counted. The remaining 25 bunches were tagged and bagged using pollinator exclusion bags to exclude bees from reaching flowers. These closed flowers were assumed to get self-pollinated in the absence of bees. Number of fruits produced, their size and weight, and the numbers of seeds in each fruit were recorded as in the previous trial.

2.7. Data Analysis. Data analysis was carried out using Microsoft Excel 2008 and Minitab version 16. Two-sample *t*-test was carried out using Minitab 16 to determine whether there is a significant difference between handling times of the two common buzzing bees at flowers of each age category and the difference between the body lengths of the two common buzzing bee species at 95% confidence interval. Difference between the fruit length and weight and number of seeds produced from fruits of the two trials was also analyzed using two-sample *t*-test.

3. Results

3.1. Species Composition and Diurnal Pattern of Bee Visitation. A total of seven bee species visited flowers of *S. melongena* at the two sites. The pollen bees, belonging to family Halictidae (*Hoplonomia westwoodi*, *Patellapis kaluterae*, and *Pachynomia* sp.) and family Apidae *(Amegilla comberi* and *Xylocopa tenuiscapa)*, buzzed at the anthers of *S. melongena* flowers. The honeybee species, *Apis cerana,* and *Trigona iridipennis* were the non-buzzing bees. *Apis cerana* collected pollen by damaging the anther base and milking the anthers and *T. iridipennis* collected pollen fallen around anther cones and on flower petals. The site at Gelioya harbored all seven species of bees, while the site at Miwatura recorded only five bee species except the two buzzing bees, *Pachynomia* sp. and *X. tenuiscapa*. Though counts of bees were not made, according to the close observations, common buzzing bees visiting flowers at Miwatura site were *H. westwoodi* and in Gelioya site they were *H. westwoodi* and *Pachynomia* sp. The activity periods of bees at the Miwatura site and Gelioya site, respectively, are shown (Figure 1) and indicate that the peak activity period of buzzing bees and nonbuzzing bees and also between the two sites is different. At Miwatura site, bees were very active between 09:00 and 12:00 hours and continued till 14:00. At Gelioya site, they were very active from 08:00 to 10:30 and continued until 11:30 and there were no bees visiting flowers after 11:30. At Gelioya site, six bee species (except *Xylocopa tenuiscapa*) were recorded from flowers daily. Buzzing bees were the first to visit *Solanum* flowers and were followed by nonbuzzing bees. Observation of the activity of individual bees at Miwatura site indicated that *H. westwoodi* was the most active bee species from 08:00 to 14:00 of the day. However, activity of this species was limited to 1-2 hours at the Gelioya site. Closer examination of the anther and stigma of flowers indicated that the stigma remains receptive from 08:00 to 11:00 in the morning and the anthers open around 08:00. This period correlated with the period of high bee activity in both sites.

3.2. Number of Pollen Grains per Anther and Handling Time of Bees with Flower Age. Solanum melongena has triporate type pollen. The amount of pollen available in the anthers of flowers of the four age groups was estimated to find out the relationship between handling time by bees and the amount of pollen available in anthers. The highest amount of pollen was available in new anthers (>200,000 grains), while the lowest amount of pollen (<50,000 grains) was found in anthers that are more than two days old, indicating a gradual decrease in the available pollen grains per anther with age of flower (Figure 2).

Two species of common buzzing bees, *Pachynomia* sp. and *H. westwoodi*, at Gelioya site were selected to study the handling time of each species at anthers of *S. melongena* flowers of different age group. The averaged handling time of *Pachynomia* sp. and *H. westwoodi* is given (Figure 3) and shows that buzzing time of *Pachynomia* sp. was highest at flowers of all four age groups compared to that of *H. westwoodi*. Results of paired *t*-test revealed that the buzzing times of the two species of bees differ significantly ($p < 0.05$) at flowers of each age group. Both species of bees buzzed for longer time on new flowers with large amount of pollen and the time decreases with the age of flower (amount of pollen). The body length of *H. westwoodi* is 8.50 mm while that of *Pachynomia* sp. is 7.00 mm, indicating that the larger *H. westwoodi* buzz at flowers for shorter periods, while the smaller *Pachynomia* sp. buzz for longer periods at flowers.

3.3. Effect of Bee Visits on Fruit Set and Seed Set. The percentage of fruit production was higher (62.5%) in open bunches of flower buds that were made available for bees to visit than in closed bunches of flower buds (18.9%). The mean length (±standard deviation) of fruits developed from closed flowers was 16.14 ± 3.29 cm and that of open flowers was 16.185 ± 1.87 cm. Therefore, the difference of the fruits in length was not significant ($p = 0.96$). Mean weight (± standard deviation) of fruits produced from closed flowers was similar to that of those produced from open flowers (122.12 ± 22.66 g and 122.37 ± 29.69 g, resp.) and was not significant ($p = 0.98$). However, the number of seeds produced by the fruits of the two treatments was significantly different ($p < 0.05$) and fruits produced from open flowers had large number of seeds (mean± = 1250 ± 265) compared to that of closed flowers (mean± = 723 ± 265).

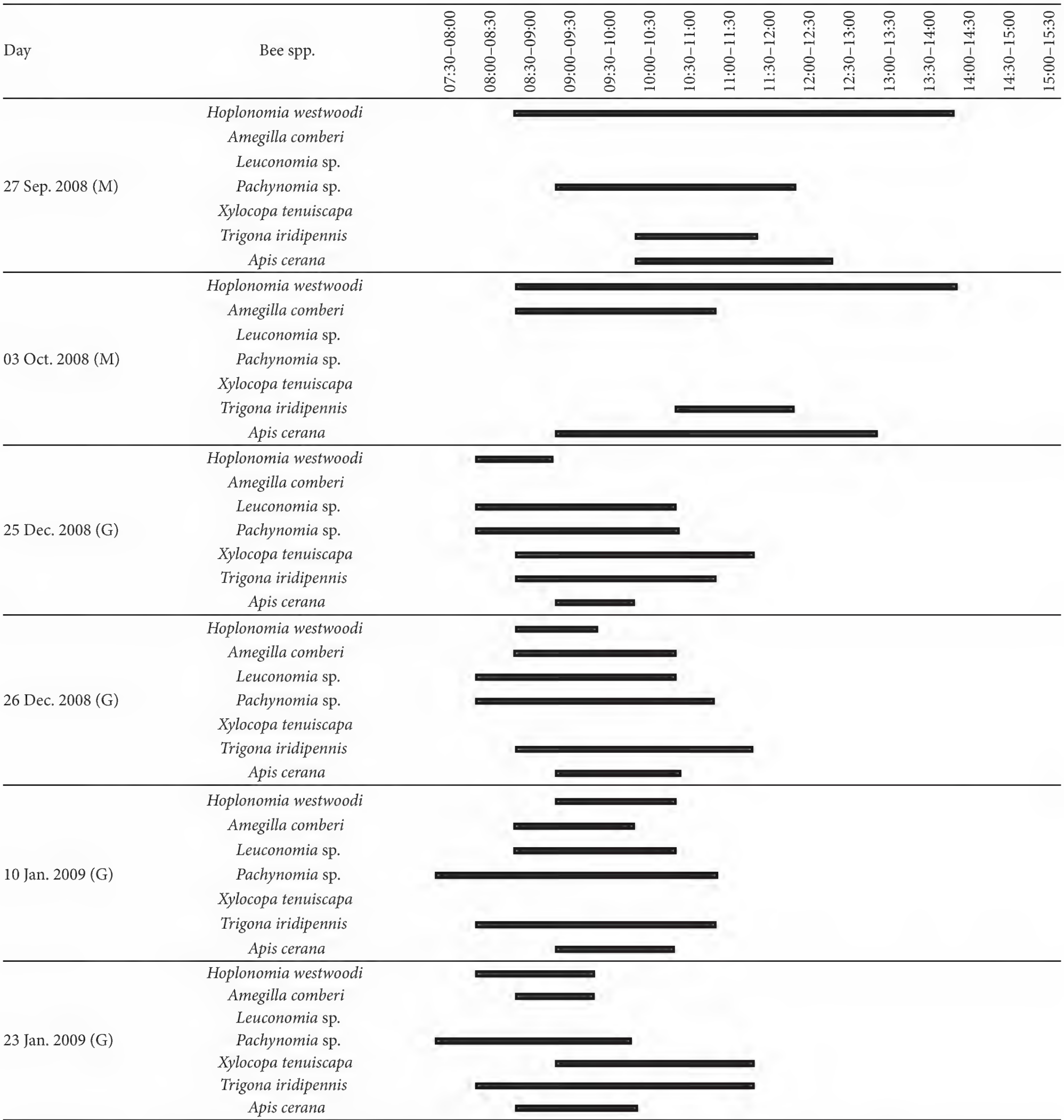

FIGURE 1: Activity period of bees at Miwatura (M) and Gelioya (G) sites in two and four sunny days, respectively, at flowers of *S. melongena*. Range in temperature (°C), rainfall (mm), and relative humidity during the time period of observations for the six days: 27 September 2008—20.80–31.80°C, 0.0 mm, and 62–65; 03 October 2008—20.00–31.70°C, 0.0 mm, and 54–61; 25 December 2008—21.40–29.50°C, 0.0 mm, and 55–57; 26 December 2008—19.40–30.80°C, 0.0 mm, and 50–53; 10 January 2009—20.90–29.80°C, 0.0 mm, and 62–65; and 23 January 2009—17.00–30.00°C, 0.0 mm, and 51–68.

4. Discussion

Although *S. melongena* flowers lack nectar and have terminal anther pores [28], they were visited by many bee species for the rich pollen resource. Studies conducted in different parts of Sri Lanka from 2001 to 2004 list 16 species of bees visiting flowers of *S. melongena* [29, 30]. During the present study, *S. melongena* flowers attracted seven species of wild bees. Among them, only five bee species buzzed at flowers. But the other two honeybees did not buzz at flowers to collect

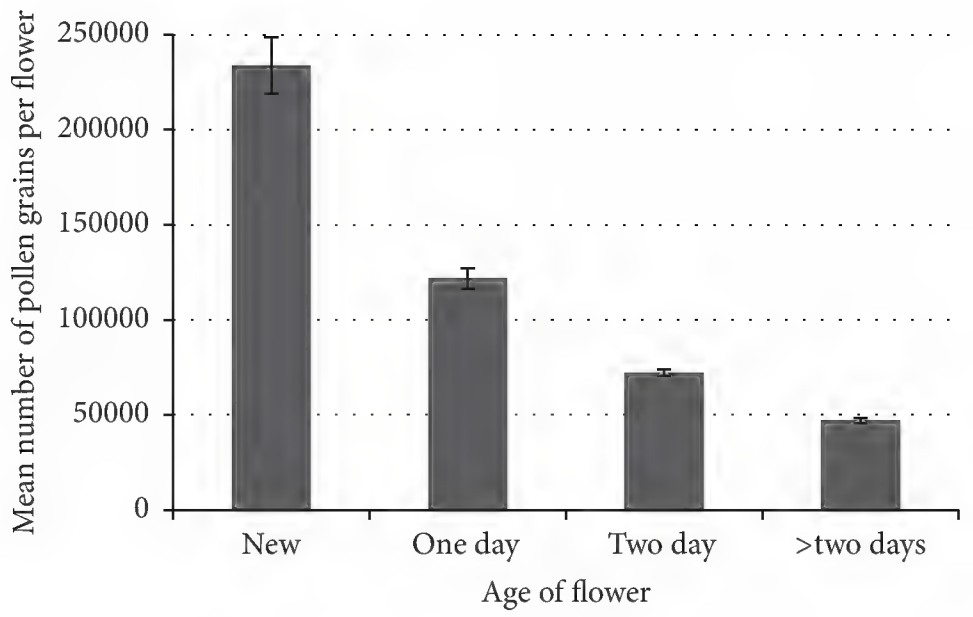

FIGURE 2: Number of pollen grains per anther with the age of flowers of different ages.

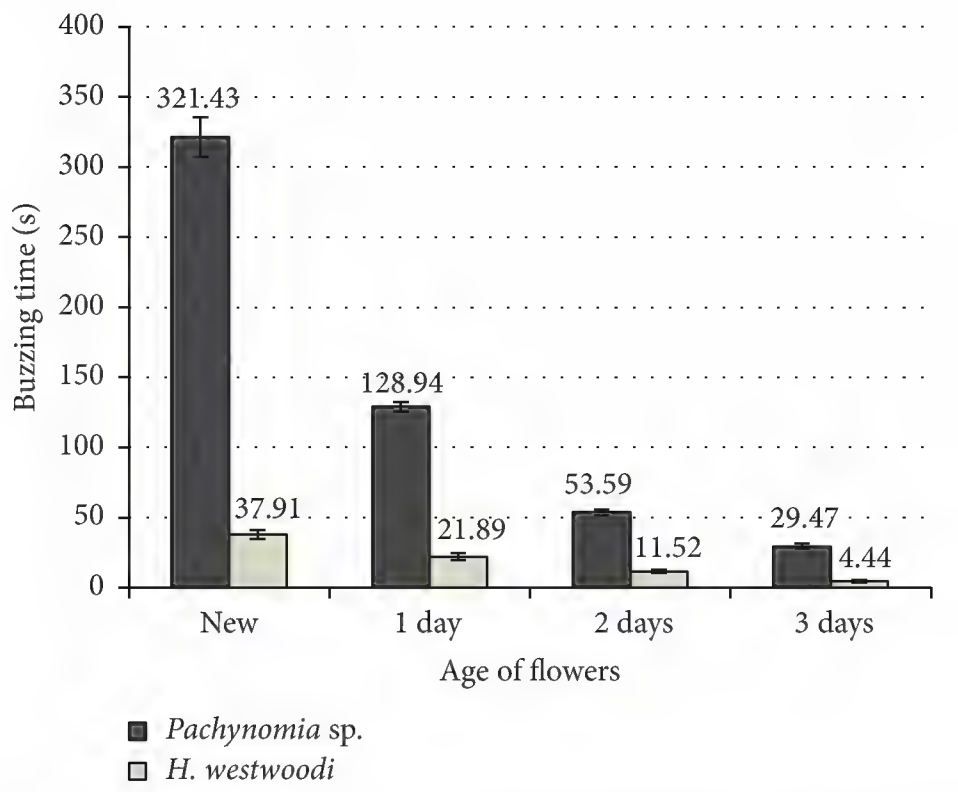

FIGURE 3: Comparison of buzzing times of *Pachynomia* sp. and *H. westwoodi* bees at *S. melongena* flowers of the four age groups (new, 1-day-old, 2-day-old, and 3-day-old flowers).

pollen from anthers [12]. Among the five buzzing bees, three belonging to family Halictidae contain the highest number of bee species of Sri Lanka. The other two buzzing bees belong to family Apidae. Buzzing bees were the first to arrive at flowers early in the morning and these were followed by nonbuzzing bees. Thereafter, both groups were found together actively collecting pollen from flowers. As observed by Wanigasekara and Karunaratne [31], buzzing by the first bee species to arrive at flowers of *S. violaceum* released pollen from the apical pores of anthers, initiating pollen release and making opened anthers available to nonbuzzing bees. The stingless bees, *Trigona iridipennis*, were seen collecting pollen fallen around the anther cones and petals. According to Anderson and Symon [28], species as *Trigona* are simply pollen thieves, although they do not pierce the flowers to get pollen. They collect pollen spread over the flowers which was released due to the activities of the vibratile pollinators and they also foraged on the stigma [28].

Among two study sites, Gelioya site contained all recorded seven species including two additional species compared to those recorded at Miwatura site. This may be because the Gelioya site is in a village setting with diverse vegetation comprising cultivated crops and natural vegetation belonging to many plant families that provide a continuous year-round supply of food for the native wild bees. In contrast, except for the area under cultivation, Miwatura site has a dense cover of the invasive grass, *Panicum maximum*, which has become very difficult to control. Absence of cultivated and natural vegetation which serves as food plants and nesting grounds for bees at this site may have resulted in paucity of bees. A previous survey of bees conducted at the Miwatura site from 2001 to 2003 [32], when the area was rich in annual and perennial broadleaf herbaceous weeds and cultivated plants, recorded five species of buzzing bees, *Amegilla comberi, Amegilla* sp., *Gnathonomia nasicana, Leuconomia* sp., and *H. westwoodi*, from *S. melongena*.

The peak activity of buzzing bees coincided with the time of anther opening and stigma receptivity enabling cross pollination of *S. melongena* flowers by bees. A similar study conducted in Southern Costa Rica under tropical weather conditions indicated a similar high bee activity period during anther dehiscence and stigma receptivity in flowers of *S. wendlandii*, which is an ornamental plant [33]. A similar field study conducted in the central Kegalle District of Sri Lanka by Wanigasekara and Karunaratne [31] also gave a similar finding. Although the findings indicate that stigma receptivity and release of pollen occur at the same time, there is a lesser chance for self-pollination as the stigma of *S. melongena* protrudes over the anther, preventing the possibility of self-pollination. But buzzing bee activity coincides with the time of stigma receptivity and release of pollen indicates the high possibility of both self-pollination and cross pollination mediated by bees.

The observation that bees buzzed longer on new flowers which decreased with increasing age of flowers was similar to study done by Shelly and Villalobos [33] for bumble bees. According to them, bees rejected older flowers with pale colours and different floral shape. The pollen-collecting bees are sensitive to the high availability of resources [31]. But, according to Burkart et al. [34], bees are unable to make long-distance assessment of pollen availability; hence, they visit new and old flowers at the same frequency during day time. Upon alighting on a flower, bees adjust the time they spend at an individual flower, based on pollen availability. These findings agree with those of the present study where both buzzing bee species showed highest handling time at new flowers compared to low handling time at flowers that are more than two days old likely due to the high pollen availability in new flower anthers compared to old flower anthers. King and Buchmann [35] stated that large amounts of pollen (72,000 grains) are released in initial sonication of new flowers and with the age of flower, the amount of pollen in flowers decreased. Harder and Barclay [36] also reported that flower's age significantly affects the removal of pollen by bees. The relationship between buzzing time and availability of pollen in anthers of different ages reported in this study agrees with most of the previous findings.

The present study further confirms the availability of large amount of pollen (200,000 grains per anther) in new flowers compared to flowers over two days old (<50,000 grains per anther). Therefore, it is evident that bees spend more time at new flowers which are rich in pollen as a cost-effective measure. But this finding is contradictory to Buchmann [12] who reported that handling time is shorter at new flowers than at older flowers.

The finding that the larger bee, *H. westwoodi*, spends less time buzzing at flowers than the smaller bee, *Pachynomia* sp., agrees with studies conducted in Australia by Anderson and Symon [28]. They reported that the larger blue banded bee, *Amegilla* sp., spent less time at flowers compared to *Nomia* sp. which is comparatively smaller. Similar observations have been made by Wanigasekara and Karunaratne [31] for *S. violaceum* flowers buzzed by larger bee, *H. westwoodi*, and a comparatively smaller bee, *Patellapis kaluterae*. This result is also equivalent to explanation done by Wanigasekara and Karunaratne [31] as vibration done by buzzing bees may singly or collectively be based on their body size. It is noteworthy that the buzzing time of *H. westwoodi* is very much similar and follows the same pattern at flowers of both *S. violaceum* and *S. melongena* irrespective of the size of flowers.

In the present study, although the number of fruits produced by open flowers was greater than that produced by closed flowers, the difference was not significant. According to Buchmann [12], bees are essential to pollinate eggplant flowers efficiently. Kakizaki [20] reported that *Solanum* plants grown in cages without bees produced no fruits. Similar observations were made by Jones and Rosa [37] and Pal and Taller [21]. Although a large amount of pollen is released onto the stigma, in self-pollination, the fruit set is low compared to buzz pollination. Findings of Duncan et al. [38] were similar for *Dianella revoluta* (Phormiaceae) which is a buzz-pollinated flower. Jones and Rosa (1928) reported that plants that were isolated from insect visitors bore no fruits. Although there was no significant difference in the size of fruits produced by open and closed flowers of *S. melongena*, the number of seeds produced per fruit in open flowers was significantly higher, indicating the importance of bees. Several studies have been carried out to test the effect of self-pollination on seed set in eggplant. McGregor [22], Bailey [23], and Aizen et al. [39] reported that a large number of seeds are produced following cross pollination by bees compared to artificial pollination. Pal and Taller [21] also stated that the number of seeds per fruit is higher in cross-pollinated flowers than in self-pollinated flowers.

The finding that buzz pollination enhances seed set in *S. melongena* is of significance to agriculture as *S. melongena* is propagated by seeds. This study, like many other similar studies, highlights the role of wild buzzing bees in the propagation of *S. melongena*, a popular vegetable crop grown extensively in Sri Lanka. Since buzzing bee species that visit *S. melongena* flowers are generalist bees, their presence can be enhanced by planting flowering plants that supply a rich source of pollen year round and providing nesting sites for ground and wood nesters in the vicinity of cultivations.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Repellent Effect and Insecticidal Activities of *Bridelia ferruginea*, *Blighia sapida*, and *Khaya senegalensis* Leaves Powders and Extracts against *Dinoderus porcellus* in Infested Dried Yam Chips

Laura Yêyinou Loko,[1] Obédatou Alagbe,[2] Elie A. Dannon,[3] Benjamin Datinon,[3] Azize Orobiyi,[1] Agnés Thomas-Odjo,[2] Alexandre Dansi,[1] and Manuele Tamò[3]

[1]*Faculty of Sciences and Technology of Dassa, Université Nationale des Sciences, Technologies, Ingéniérie et Mathématiques d'Abomey, BP 14, Dassa, Benin*
[2]*Faculty of Agronomy, University of Parakou, BP 123, Parakou, Benin*
[3]*International Institute of Tropical Agriculture, 08 BP 0932, Cotonou, Benin*

Correspondence should be addressed to Laura Yêyinou Loko; lokoestelle@yahoo.fr

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Dinoderus porcellus is considered as the most important pest of stored yam chips and compounds extracted from plants can be used for its control. The present study aimed to test the insecticidal and repellent activities of powders and extracts of leaves of *Bridelia ferruginea*, *Blighia sapida*, and *Khaya senegalensis* against *D. porcellus*. The efficacy of plant powders was compared with the synthetic pesticide Antouka (Permethrin 3 g/kg + pirimiphos 16 g/kg). The results of the experiment revealed that all plant powders were effective as repellents. Antouka was more effective as insecticidal than the plant powders and minimal weight loss was observed with *B. sapida* at 2%. Among treatments, propanol extract of *K. senegalensis* at 5% was found to elicit the highest repellent effect on *D. porcellus*. The LC_{50} results revealed that the acetone extract of *K. senegalensis* is the most toxic (0.29 μL/insect) to the pest, while the propanol extract of *B. ferruginea* at 5% exhibited strong fumigant toxicity against *D. porcellus*, with 88.89% of pest mortality at 160 μL/L air. The findings from the current work proved that plant powders and extracts of the three plants are sources of botanical insecticides which may be used in the integrated management of *D. porcellus*.

1. Introduction

Yam (*Dioscorea* spp.) is an important crop contributing to food security and poverty alleviation in sub-Sahara region, especially in West Africa [1]. In Benin, dehydrated yam chips are the only form in which fresh yam tubers are preserved throughout the year [2]. "Télibo" or "Amala," the traditional thick paste obtained from yam chips flour, is the staple food of many people in Benin [3]. Unfortunately, dried yam chips in traditional storage systems are severely attacked by the beetle *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae) which rapidly reduces yam chips into powder within few days of storage [3, 4]. This pest causes heavy qualitative and quantitative losses during storage [2, 4, 5]. Synthetic insecticides are currently used by farmers to control this pest [2], leading to many cases of food poisoning [6, 7]. The use of these insecticides also leads to a number of problems, such as danger of pesticide misuse, killing of nontarget species, toxicity residues in food, insect resistance, and the destruction of the balance of the ecosystem [8]. Therefore, there is an urgent need to develop an alternative control method that preserves human health and the environment.

Alternatives to these synthetic chemicals are extracts or powders from of some plants [9]. In fact, plants contain bioactive metabolites, which act as antifeedants, repellents, and toxicants against a wide range of insects that attack stored

products [10]. In addition, these indigenous plants, which are used as crude materials to control insect pest infestations, are harvested locally, are cheap, and require only limited processing [11]. In Benin, several medicinal plants such as *Bridelia ferruginea*, *Blighia sapida*, and *Khaya senegalensis* are used by women to control the dried yam beetle *D. porcellus* [2]. In fact, the antifungal [12], antibacterial [13], and insect antifeedant [14] properties of leaf extract of *K. senegalensis* have been demonstrated. The fruit of ackee *(B. sapida)* has insecticide properties [15], whereas the antimicrobial properties of leaves and bark extract of *B. ferruginea* have been proved by Sahu et al. [16]. In order to develop an integrated approach to control and lay the groundwork for the development of a botanical insecticide, experiments should be carried out to assess the insecticidal and repellent effects of these three plants species on *D. porcellus*. In the present study, crude powders and five extracts (acetone, ethanol, methanol, propanol, and distilled water) of *B. ferruginea*, *B. sapida*, and *K. senegalensis* leaves were evaluated for their repellent and insecticidal effects on *D. porcellus*.

2. Material and Methods

2.1. Rearing of Dinoderus porcellus. The beetle *D. porcellus* was reared using healthy yam chips as described by Onzo et al. [17]. Yam chips were sterilized in an oven at 105°C for 2 hours to kill hidden insects and their eggs. The rearing material used is made of plastic containers (19.5 cm height, 6.5 cm diameter). The plastic containers were kept in the laboratory under temperature conditions of 25 ± 2°C, relative humidity of 70 ± 5%, and photoperiodicity of 12L/12D [5]. The containers were placed on shelves in the laboratory. Every two weeks, adult insects were removed in order to synchronize the F1 progeny used for experiments [18].

2.2. Plant Materials. The fresh leaves of *B. ferruginea*, *B. sapida*, and *K. senegalensis* were collected in the town of Parakou (latitude: 9°20′13″N and longitude: 2°37′49″E). Their identity was confirmed by the National Herbarium of the University of Abomey-Calavi. The leaves were washed with tap water to remove debris. The clean leaves were dried during seven days at the room temperature in the shade to prevent the degradation of bioactive compounds by sunlight. The dried leaves were ground into fine powder with an electrical blender and sieved to obtain the finest particles using a 300 μm sieve. The fine powder obtained from each plant species was packed in an airtight container and stored in a cool dry place until use.

2.3. Repellent Activity of Leaf Powder. A bioassay consisting of a circular flat-bottomed plastic basin (26 cm in diameter by 3 cm in height), whose base was divided into five equal portions as described by Ogendo et al. [19], was used to evaluate the repellency of crude *B. ferruginea*, *B. sapida*, and *K. senegalensis* powders against adult *D. porcellus*. Each plant powder was evaluated at four rates (2, 5, 7, and 10% w/w) and commercial synthetic insecticide Antouka (Permethrin 3 g/kg + pirimiphos 16 g/kg; DP) included as a positive control. Treated and untreated yam chips (10 g) were alternately placed equidistantly from the center of the circular base [20]. The treatments were arranged in a completely randomized block design with four replicates per concentration. For each treatment, 20 starved (1 hour of starvation) adults of *D. porcellus* (3–7 days) were released at the center of the basin, which was immediately covered with a transparent muslin, in order to prevent the insects from escaping to the external environment [18]. The total number of insects that settled on the control (P) and the treated yam chips (G) was recorded after 1, 12, and 24 h of exposure. According to Dutra et al. [21], the repellency activity of plant was estimated by calculating the percent repellency (PR) and repellency index (RI). Percent repellency (PR) was calculated using the formula of McDonald et al. [22]:

$$\mathrm{PR} = \left[\frac{(N_c - N_t)}{(N_c + N_t)}\right] \times 100, \tag{1}$$

where N_c is the number of insects on untreated yam chips; N_t is the number of insects on treated yam chips. The mean repellency value of each extract was calculated and assigned to repellency classes from 0 to V: class 0 (PR ≤ 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (40.1–60%), class IV (60.1–80%), and class V (80.1–100%).

The repellency index (RI) was calculated with the formula:

$$\mathrm{RI} = \frac{2G}{G} + P, \tag{2}$$

where G is the percentage of insects attracted to the treatment and P is the percentage attracted to the control. The RI values range between zero and two [23], and RI = 1 indicates similar repellency between the treatment and the control (neutral treatment), RI > 1 indicates lower repellency of the treatment compared to the control (attractive treatment), and RI < 1 corresponds to a greater repellency of the treatment compared to the control (repellent treatment) [24].

2.4. Contact Toxicity of Plant Powders. The experiment was carried out following the methodology used by Chebet et al. [20]. Leaf powders of the different plants were admixed with 100 g of disinfected yam chips in plastic jars (13 cm in diameter by 10 cm in height) at, respectively, rates (% w/w) of 0, 2, 4, 6, 8, and 10. Yam chips treated with synthetic insecticide Antouka (0.05% w/w) were used as positive control. Ten pairs of adult unsexed insects (3 to 7 days old) were introduced into treated and untreated yam chips. Each jar was covered with a muslin cloth to prevent insects from escaping to the external environment. A completely randomized design with 4 replicates per treatment was used. Data on insect mortality was taken at 1, 3, 5, 7, 14, and 21 days after exposure [25]. The percentage adult mortality was computed according to Asawalam et al. [26] and corrected with Abbott's formula [27] to eliminate natural mortality of control.

$$\text{Percent mortality} = \frac{\text{Number of dead } \textit{D. porcellus}}{\text{Number of introduced } \textit{D. porcellus}} \times 100. \tag{3}$$

$$\text{Corrected mortality} = \frac{(\%\text{ of death in treated} - \%\text{ death in control})}{(100 - \%\text{ death in control})} \times 100. \quad (4)$$

Any living adult insect was removed on day 21 and the percentage weight loss was calculated using the formula

$$\text{Percentage weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (5)$$

For determination of F1 progeny emergence, yam chips were checked for adult emergence 35 days after exposure [5] and every 2 days thereafter. In order to avoid a second generation, the sample inspections continued until no more adults emerged during three consecutive inspections [28]. The percent reduction in adult emergence or reproduction inhibition rate (IR%) was computed according to Tapondjou et al. [29] using the formula

$$\text{Reproduction inhibition rate } (\%) = \frac{N_C - N_T}{N_C} \times 100, \quad (6)$$

where N_C is the number of newly emerging adult insects in the untreated control and N_T is the number of newly emerging adult insects in the treated yam chips.

2.5. Preparation of Plant Extracts. The extracts were prepared according to Mansoor-ul-Hasan et al. [30]. Fifty grams of each plant was taken in a beaker separately and mixed with 100 ml of different solvents (acetone, ethanol, methanol, propanol, and distilled water). Then the mixture was stirred for 30 min by a magnetic stirrer (at 6000 rpm) and left to stand for the next 24 hours [31]. The extract was sieved through Whatman filter paper to remove particles. After filtration, the acetone, ethanol, methanol, and propanol extracts were left to evaporate at room temperature during 48 h and the aqueous extract was evaporated under vacuum at 100°C [32]. The extracts were stored at 4°C until further analysis.

2.6. Preparation of Different Concentrations. By diluting the condensed extracts with acetone, ethanol, methanol, propanol, and distilled water, the stock solutions of plant extracts were prepared. Three different concentrations, namely, 2.5, 5.0, and 7.5% of each category of plant extracts were prepared by dissolving the stock solution in the respective solvent [31].

2.7. Repellent Activity Bioassay. The repellency was tested according to Hamouda et al. [32]. Half filter paper discs (Whatman number 40, 9 cm diam.) were prepared and a volume of 200 μl of each plant extract concentration was applied separately to one-half of the filter paper as uniformly as possible with a micropipette. The other half (control) was treated with 200 μl of different solvent (acetone, propanol, ethanol, methanol, and distilled water). Both the treated and the control halves were allowed to dry out as exposed in the air for 10 min. Each treated half disc was then attached lengthwise, edge to edge, to a control half disc with adhesive tape and placed in a Petri dish (9 cm diameter) [32]. Twenty adult insects were released in the middle of each filter paper circle. Each concentration was replicated four times. Insects that settled on each half of the filter paper disc were counted after 15 min, 30 min, and 2 h. The average of the counts was converted to percentage repellency (PR) using the formula of McDonald et al. [22] as in (1).

2.8. Contact Toxicity by Topical Application. Bioassays were conducted according to the method described by Aryani and Auamcharoen [33]. With the help of a pipette, 1 μL solution of each plant extract concentration was applied on the thorax of 10 adults. The control received 1 μL of each solvent (five replications). Each group of ten treated insect individuals was then transferred into a small plastic cup of 8 cm diameter and 5 cm height containing 10 g of yam chips. This experiment was replicated four times and arranged in completely randomized design (CRD). The mortality was recorded after 2, 7, 14, and 21 days [32, 33]. The percentage adult mortality was computed according to Asawalam et al. [26] and corrected for natural mortality using Abbott's formula [27] as in (3) and (4), respectively. Any living adult insect was removed on day 21 and the percentage weight loss was calculated as in (5).

2.9. Fumigation Toxicity Bioassay. Fumigant toxicity of the five solvent extracts of *B. ferruginea*, *B. sapida*, and *K. senegalensis* leaves at the three different concentrations (2.5, 5.0, and 7.5%) was assessed following Nattudurai et al. [34]. An aliquot of 0, 5, 10, 20, and 40 μL of each solvent extract was evenly applied to Whatman number 1 filter paper strips (2 cm diameter) corresponding to the dosages of 0 (as a control), 20, 40, 80, and 160 μL/L air [34]. Each treated paper strip was attached inside the cap of 50 ml glass bottle (4.5 cm in diameter by 13 cm in height) that contained 10 g of yam chips. After release of 10 adult insects the glass bottles were closed airtight by the caps connected to treated paper. After 24 h of treatment, the insects were observed and when there was no leg or antennal movements, insects were considered dead. The experiment was conducted in completely randomized design, with nine treatments and four replicates. Percent insect mortality was calculated and corrected by Abbott's formula [27]. Toxicity ratios (TR) were calculated using the formula used by Gusmão et al. [23]:

$$\text{Toxicity ratios (TR)} = \frac{\text{LC}_{50}\text{ of the extract with less toxicity}}{\text{LC}_{50}\text{ of the other extracts individually}}. \quad (7)$$

2.10. Statistical Analysis. Mortality values were corrected with Abbott's formula to eliminate natural mortality of control. Data on percentage mortality and repellency were arcsine transformed, in order to homogenize their variance before being subjected to one-way ANOVA using IBM SPSS Statistic Software Version 23.0. Significant differences

between means were separated using Least Significant Difference (LSD) at 5% probability. Back-transformed (original) data are given in tables and figures. Data obtained from various concentration-response bioassays (contact toxicity and repellence) were further arcsine transformed before being subject to probit regression analysis using IBM SPSS Statistic Software Version 23.0. Probit analysis was used to calculate the median repellent dose RD_{50} (dose that repelled 50% of the exposed insects). The relationship between the extract concentration applied and percentage mortality was determined using probit regression analysis of transformed data to estimate lethal concentration that kills 50% (LC_{50}) of test insects. Any two LC_{50} values in a column whose 95% confidence limits did not overlap were regarded as significantly different.

3. Results

3.1. Repellent Effect of Plant Powders. Percent repellency of *D. porcellus* adults to yam chips treated with the various crude powder concentrations of *B. ferruginea, B. sapida, and K. senegalensis* is given in Table 1. The magnitude of *D. porcellus* adult repellency was not significantly influenced by plant species ($F = 0.341$, dl = 2, $p \geq 0.05$), concentration of powder applied ($F = 1.477$, dl = 3, $p \geq 0.05$), and exposure time ($F = 1.461$, dl = 2, $p \geq 0.05$). The synthetic insecticide Antouka and all the plant powder possess repellent activity against *D. porcellus* (Table 1). However, repellency of the plant powders was not dose-dependent. The mean percentage repellency value reached 51.3% at the dose of 7% of *K. senegalensis* powder within 24 h of exposure (Table 1). Based on mean repellency rate, powders of all three plants showed repellency classes II and III. The RD_{50} values indicate that synthetic insecticide Antouka repelled *D. porcellus* better than any other plant powders 1 and 12 hours after treatment with a RD_{50} value of 5.4% and 5.9% (w/w), respectively (Table 1), while, 24 hours after treatment, *K. senegalensis* powder was more repellent than the other treatments with a RD_{50} value of 4.1% (w/w).

3.2. Insecticidal Efficiencies of Plant Powders. Contact action of *B. ferruginea, B. sapida*, and *K. senegalensis* leaves powders against adult *D. porcellus* is presented in Table 2. The findings of this experiment revealed that insect mortality on yam chips admixed with powders varied with the dosage, the plant tested, and the exposure time (Table 2). The mortality effects of the plant powders on *D. porcellus* were significantly different from the effect of the synthetic insecticide Antouka after 1 day ($F = 2.084$, dl = 15, $p \leq 0.001$), 3 days ($F = 3.831$, dl = 15, $p \leq 0.001$), 5 days ($F = 4.768$, dl = 15, $p \leq 0.001$), and 7 days ($F = 2.260$, dl = 15, $p \leq 0.001$) of exposure. Commercial product Antouka was most effective at all exposure periods and caused 100% mortality of *D. porcellus* 7 days after treatment. However, any significant difference was not observed between synthetic insecticide and plant powders 14 days ($F = 0.797$, dl = 15, $p \geq 0.05$) and 21 days ($F = 0.545$, dl = 15, $p \geq 0.05$) after treatment (Table 2). After 1 day of exposure, a dosage of 2, 4, and 6% (w/w) of *B. sapida* and 8% of *K. senegalensis* powders was not significantly different from that of synthetic insecticide Antouka in terms of *D. porcellus* mortality (Table 2). The same trend was observed with *B. sapida* at 4 and 6%, respectively, 7 and 3 days after treatment (Table 2). Although none of the tested plant powder was able to exert 100% adult mortality, *B. sapida* 4% (w/w) caused 43.7% adult mortality at 21 days after infestation (Table 2). In general, mortality of *D. porcellus* caused by the various concentrations of the three leaf powders tested was higher than those of the control after 14 days of treatment.

The weight loss of yam chips caused by the feeding activity of *D. porcellus* varied in function of plant species and dosage (Table 2). The data recorded at 21 days after treatment registered no weight loss in yam chips treated by the synthetic insecticide Antouka. The highest mean weight loss was 6.7 ± 1.1 g on the untreated control (Table 2). The *K. senegalensis* leaves powder at 8% recorded a significantly ($F = 2.394$, dl = 16, $p \leq 0.001$) higher weight loss when compared to the other treatment (Table 2). Among the plant powder treatments, minimal weight loss was observed in the treatment with *B. sapida* powder at 2% (Table 2).

The adult *D. porcellus* F1 progeny counts in yam chips treated with crude botanical powders were not significantly affected by the plant species ($F = 0.848$, dl = 2, $p \geq 0.05$) and concentration applied ($F = 0.135$, dl = 4, $p \geq 0.05$). Synthetic insecticide Antouka, at 0.05% (w/w), caused the total inhibition (100%) of adult *D. porcellus* F1 progeny emergence. The median developmental time of *D. porcellus* varied significantly ($F = 2.845$, df = 16, $p \leq 0.001$) from 0 days for yam chips treated with synthetic insecticide Antouka to 37.6 days for a dosage of *B. ferruginea* at 10% (Table 3). The reproduction inhibition rate was maximum (100%) in synthetic insecticide Antouka during storage at 21 days after treatment. It was followed by *B. ferruginea* at 4 (50%), 6, and 10% with, respectively, 45% of inhibition rate. In contrast, the *B. sapida* powder at 4 and 10% registered low inhibition rate, that is, 5%, respectively (Table 3).

3.3. Repellency of Plant Extracts. Results given in Table 4 describe the repellent activity of different solvents and concentrations of *B. ferruginea*, *B. sapida*, and *K. senegalensis* leaves crude extracts. The repellency rate of acetone, propanol, ethanol, methanol, and distilled water solvents extract of three plants showed insignificance at different time after treatment (Table 4). The results revealed that repellent activity did not happen significantly at 15 min ($F = 0.822$, df = 44, $p \geq 0.05$), 30 min ($F = 0.926$, df = 44, $p \geq 0.05$), and 2 h ($F = 0.854$, df = 44, $p \geq 0.05$) after treatment. However, even though almost all the plant crude extracts did not show the repellent potential almost at the first 30 min, but as time progressed, the level of repellent activity was increased (Table 4). Among all three plant species and five solvents tested, propanol extract of *K. senegalensis* at 5% was found to elicit significantly ($F = 1.871$, df = 44, $p \leq 0.001$) the highest repellent effect on *D. porcellus* with a percentage repellency of 30% (class II). It was followed by the aqueous extract at 7.5% and propanol extract at 2.5% of *B. sapida* with, respectively, 26.9% (class II) and 22.7% (class II) repellency of *D. porcellus*. Repellency index ranged from 0.7 to 1.2 (Table 4). The acetone extract of *B. ferruginea*, *B.*

Table 1: Percent repellence (mean ± SE) of adult *D. porcellus* and repellent class of *B. ferruginea*, *B. sapida*, and *K. senegalensis* crude powders to varying exposure time and concentrations in a choice bioassay.

Treatments	Concentration (% w/w)	Percent repellency at different time intervals			Mean repellency	Repellency class	Repellency index	Classification
		1 h	12 h	24 h				
Antouka	2.0	35.4 ± 15.8[a]	46.6 ± 9.7[a]	37.7 ± 17.2[a]	39.9 ± 7.7[a]	II	0.60 ± 0.07	Repellent
	5.0	69.0 ± 18.0[a]	37.6 ± 8.8[a]	35.0 ± 13.7[a]	47.2 ± 8.6[a]	III	0.56 ± 0.05	Repellent
	7.0	28.8 ± 41.8[a]	27.8 ± 18.4[a]	52.1 ± 14.9[a]	36.2 ± 14.9[a]	II	0.62 ± 0.06	Repellent
	10.0	75.0 ± 25.0[a]	16.8 ± 18.5[a]	28.8 ± 10.9[a]	40.2 ± 12.5[a]	III	0.61 ± 0.05	Repellent
RD_{50}		5.4	5.9	7.0				
B. ferruginea	2.0	18.9 ± 13.1[a]	42.9 ± 27.4[a]	35.8 ± 18.9[a]	32.5 ± 11.2[a]	II	0.63 ± 0.04	Repellent
	5.0	53.5 ± 18.5[a]	42.1 ± 7.8[a]	43.4 ± 8.4[a]	46.3 ± 6.7[a]	III	0.60 ± 0.04	Repellent
	7.0	51.8 ± 18.3[a]	27.3 ± 9.4[a]	42.0 ± 14.1[a]	40.4 ± 8.1[a]	III	0.61 ± 0.03	Repellent
	10.0	40.0 ± 31.9[a]	15.0 ± 14.0[a]	45.4 ± 8.7[a]	33.5 ± 11.5[a]	II	0.62 ± 0.03	Repellent
RD_{50}		9.6	8.3	7.8				
B. sapida	2.0	27.5 ± 7.5[a]	31.2 ± 18.0[a]	19.8 ± 14.2[a]	26.1 ± 7.4[a]	II	0.63 ± 0.03	Repellent
	5.0	29.9 ± 27.1[a]	47.9 ± 16.8[a]	45.8 ± 11.0[a]	41.2 ± 10.5[a]	III	0.63 ± 0.03	Repellent
	7.0	39.6 ± 15.7[a]	39.1 ± 8.4[a]	41.1 ± 19.3[a]	39.9 ± 7.9[a]	II	0.62 ± 0.02	Repellent
	10.0	53.6 ± 27.0[a]	35.4 ± 20.5[a]	51.3 ± 20.0[a]	46.8 ± 12.1[a]	III	0.61 ± 0.02	Repellent
RD_{50}		9.6	11.6	7.6				
K. senegalensis	2.0	25.0 ± 14.4[a]	25.5 ± 12.3[a]	37.9 ± 11.6[a]	29.4 ± 6.9[a]	II	0.62 ± 0.02	Repellent
	5.0	29.5 ± 30.9[a]	34.5 ± 16.1[a]	42.8 ± 11.7[a]	35.6 ± 11.2[a]	II	0.62 ± 0.02	Repellent
	7.0	25.0 ± 15.0[a]	55.3 ± 18.3[a]	73.7 ± 9.0[a]	51.3 ± 9.7[a]	III	0.60 ± 0.02	Repellent
	10.0	16.9 ± 6.9[a]	47.0 ± 11.3[a]	53.1 ± 6.7[a]	39.0 ± 6.5[a]	II	0.60 ± 0.02	Repellent
RD_{50}		17.9	8.7	4.1				
F		1.507	0.567	0.543	0.896			
p value		0.159	0.880	0.900	0.547			

Means within the same rows followed by the same letter are not significantly different ($p < 0.05$). RD_{50} refers to the concentration (% w/w) that repels 50% of the test insects using probit regression analysis.

Table 2: Mortality of adult *D. porcellus* and weight loss (mean ± SE) of yam chips treated with varying concentration of *B. ferruginea*, *B. sapida*, and *K. senegalensis* crude powders in 21 days after infestation. Mortality rate was corrected using Abbott's formula (4).

Treatments	Concentration (% w/w)	Percentage of mortality rate after different periods of exposures (days)						Weight loss (g)
		1	3	5	7	14	21	
Antouka	0.05	29.4 ± 9.9^{a}	79.1 ± 1.3^{a}	96.8 ± 3.1^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	0.0 ± 0.0^{c}
B. ferruginea	2.0	1.2 ± 2.4^{d}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{c}	5.3 ± 20.1^{a}	8.3 ± 26.4^{a}	3.0 ± 0.4^{abc}
	4.0	0.0 ± 0.0^{d}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}	1.7 ± 15.8^{c}	12.5 ± 21.5^{a}	8.3 ± 29.5^{a}	2.0 ± 0.7^{abc}
	6.0	1.2 ± 2.4^{d}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{c}	10.7 ± 10.3^{a}	18.7 ± 18.1^{a}	2.9 ± 0.7^{abc}
	8.0	2.5 ± 2.0^{bcd}	2.7 ± 4.8^{c}	0.0 ± 5.3^{cd}	3.4 ± 2.8^{b}	32.1 ± 12.2^{a}	41.7 ± 14.8^{a}	2.3 ± 0.6^{abc}
	10.0	1.8 ± 3.8^{d}	0.0 ± 0.0^{c}	0.0 ± 4.4^{d}	1.7 ± 1.7^{b}	17.8 ± 8.5^{a}	29.2 ± 15.4^{a}	2.7 ± 0.6^{abc}
B. sapida	2.0	6.4 ± 2.4^{abcd}	8.3 ± 6.6^{b}	10.9 ± 10.3^{bc}	15.5 ± 17.0^{b}	33.9 ± 19.9^{a}	27.1 ± 24.6^{a}	0.4 ± 0.1^{bc}
	4.0	11.5 ± 3.8^{abc}	11.1 ± 3.9^{bc}	20.3 ± 8.2^{b}	25.9 ± 10.3^{ab}	37.5 ± 17.1^{a}	43.7 ± 21.3^{a}	1.9 ± 0.9^{abc}
	6.0	11.5 ± 2.4^{ab}	19.4 ± 5.3^{ab}	15.6 ± 5.9^{bc}	15.5 ± 7.1^{b}	26.8 ± 6.3^{a}	22.9 ± 10.4^{a}	4.1 ± 1.6^{ab}
	8.0	2.5 ± 2.0^{bcd}	2.7 ± 1.6^{c}	1.5 ± 1.5^{d}	1.7 ± 8.6^{b}	21.4 ± 17.7^{a}	25.0 ± 22.0^{a}	4.1 ± 1.9^{abc}
	10.0	1.2 ± 1.2^{cd}	0.0 ± 2.2^{c}	4.6 ± 4.6^{bcd}	13.7 ± 4.4^{b}	28.5 ± 5.0^{a}	27.0 ± 7.8^{a}	4.1 ± 2.0^{abc}
K. senegalensis	2.0	2.5 ± 2.9^{bcd}	1.3 ± 7.3^{b}	0.0 ± 10.2^{bcd}	0.0 ± 8.8^{b}	10.7 ± 21.1^{a}	0.0 ± 26.6^{a}	3.6 ± 2.0^{abc}
	4.0	1.2 ± 1.2^{cd}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{c}	5.3 ± 15.3^{a}	4.1 ± 21.7^{a}	1.5 ± 0.5^{abc}
	6.0	2.5 ± 5.1^{d}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}	0.0 ± 11.5^{bc}	10.7 ± 19.0^{a}	10.4 ± 24.2^{a}	2.6 ± 1.2^{abc}
	8.0	10.2 ± 7.4^{abcd}	11.1 ± 9.8^{b}	3.1 ± 11.0^{bcd}	0.0 ± 13.2^{bc}	8.9 ± 25.3^{a}	4.1 ± 33.1^{a}	6.4 ± 2.2^{a}
	10.0	0.0 ± 1.3^{d}	0.0 ± 0.0^{c}	0.0 ± 0.0^{d}	6.9 ± 1.9^{bc}	16.0 ± 3.4^{a}	22.9 ± 10.4^{a}	3.9 ± 1.7^{abc}
Control	0	2.5 ± 1.4	10.0 ± 2.8	20.0 ± 6.1	27.5 ± 2.5	30.0 ± 2.0	40.0 ± 3.5	6.7 ± 1.1^{a}

Means in a column followed by different letters are significantly different at $\alpha = 0.05$.

Table 3: Effect of treating yam chips with crude powders of *B. ferruginea*, *B. sapida*, and *K. senegalensis* on F1 progeny emergence and median development time (mean ± SE) of *D. porcellus*.

Treatments	Concentration (% w/w)	Mean number of F1 progenies	Median development time	Reproduction inhibition rate (%)
Control	0.0	5.0 ± 1.9^a	27.7 ± 9.2^b	0.0
Antouka	0.05	0.0 ± 0.0^a	0.0 ± 0.0^a	100.0
B. ferruginea	2.0	3.2 ± 1.6^a	27.6 ± 9.2^b	35.0
	4.0	2.5 ± 1.3^a	27.7 ± 9.7^b	50.0
	6.0	2.7 ± 1.1^a	36.4 ± 0.5^b	45.0
	8.0	3.7 ± 1.2^a	36.4 ± 0.4^b	25.0
	10.0	2.7 ± 0.7^a	37.6 ± 0.4^b	45.0
B. sapida	2.0	3.0 ± 1.2^a	27.7 ± 9.2^b	40.0
	4.0	4.7 ± 2.2^a	36.5 ± 0.5^b	5.0
	6.0	3.7 ± 0.8^a	35.9 ± 0.1^b	25.0
	8.0	3.5 ± 0.8^a	36.2 ± 0.4^b	30.0
	10.0	4.7 ± 1.5^a	36.7 ± 0.6^b	5.0
K. senegalensis	2.0	4.0 ± 1.2^a	36.4 ± 0.5^b	20.0
	4.0	4.2 ± 1.1^a	37.2 ± 0.3^b	15.0
	6.0	4.2 ± 2.1^a	27.1 ± 9.0^b	15.0
	8.0	4.5 ± 1.7^a	37.1 ± 0.6^b	10.0
	10.0	3.7 ± 1.4^a	27.5 ± 9.1^b	25.0

Means in a column followed by different letters are significantly different at $\alpha = 0.05$.

sapida, and *K. senegalensis* at 7.5%, the methanol extract of *B. ferruginea* at 2.5%, and the ethanol extract of *B. sapida* at 7.5% and *K. senegalensis* at 2.5 and 5% were attractant for *D. porcellus* (Table 4). Considering solvents used in this study, repellency of the aqueous extract of *B. sapida* was dose-dependent, comparable to other extracts (Table 4). The comparison among solvents indicates that distilled water and propanol as a solvent were more efficient in extracting bioactive compounds even though the other three solvents also produced some effects (Table 4).

3.4. Contact Toxicity by Topical Application of Plant Extracts. Table 5 summarizes results on the toxicity of the various plant extracts applied topically to *D. porcellus*. The extracts of *B. ferruginea*, *B. sapida,* and *K. senegalensis* caused significant mortality of adult *D. porcellus*. Mortality varied between treatments, concentration, and exposure-period (Table 5). There was a significant ($F = 2.531$, df = 44, $p \leq 0.001$) difference among the extracts 2 days after treatments with acetone extract of *K. senegalensis* at 2.5% inducing the highest mortality of *D. porcellus* and aqueous extract of *B. sapida* at 2.5% the lowest mortality (Table 5). Acetone extract of *B. ferruginea* at 7.5% was significantly most effective at 7 days ($F = 2.984$, df = 44, $p \leq 0.001$) and 14 days ($F = 3.210$, df = 44, $p \leq 0.001$) after treatment while aqueous extract of *B. sapida* at 2.5% was the least. Within 21 days after treatment, aqueous extract of *B. sapida* at 2.5% was significantly ($F = 3.602$, df = 44, $p \leq 0.001$) less toxic to *D. porcellus* compared with the other extracts (Table 5). The LC_{50} ranged from 0.29 μL/insect for acetone extract of *K. senegalensis* to 34.73 μL/insect for methanol extract of *B. ferruginea* (Table 6). According to Table 6, based on the LC_{50}, acetone solvent was more efficient in extracting bioactive compounds of *B. ferruginea* and *K. senegalensis* while for *B. sapida* it was propanol. Table 5 shows that the weight loss caused by the feeding activity of adult *D. porcellus* was significantly ($F = 3.217$, dl = 45, $p \leq 0.001$) lower in the yam chips treated with methanol extract of *B. ferruginea* leaves at 7.5% compared with the other treatments.

3.5. Fumigant Toxicity. Fumigant efficacy tests of the plant extracts showed variable toxicity to adults of *D. porcellus*, depending on plant species and solvents (Figure 1). In all cases, considerable differences in mortality of insects to vapors of extracts were observed with different concentrations (Figure 1). From the graph in Figure 1, it can be seen that propanol extracts of plants were relatively more toxic to *D. porcellus* than the others. The highest concentration (160 μL/L air) of the propanol extract of the three tested plants proved able to induce more than 50% mortality 24 h after treatment (Figure 1). Propanol extract of *B. ferruginea* at 5% exhibits a fumigant toxicity of 88.89% of mortality for a concentration of 160 μL/L air (Figure 1), followed by propanol extract of *B. ferruginea* at 7.5% and propanol extract of *K. senegalensis* at 2.5% causing up to, respectively, 75.56 and 74.48% of pest mortality at the highest concentration. In fumigation tests, LC_{50} ranged from 5.40 to 194.01 μL/L air, while the toxicity ratios ranged from 1.07 to 35.92 (Table 7). Probit analysis showed that *D. porcellus* was more susceptible to propanol extracts of *K. senegalensis* ($LC_{50} = 5.40$ μL/L air) and *B. sapida* ($LC_{50} = 18.68$ μL/L air) which have a toxicity ratio of 35.92 and 10.38, respectively (Table 7).

4. Discussion

Results reported in the current study show that leaves powders of *B. ferruginea*, *B. sapida,* and *K. senegalensis* have repellent effects on *D. porcellus*. The observed repellent activity could partly be attributed to the presence of volatile

Table 4: Repellency rate of different solvent extracts of *B. ferruginea*, *B. sapida*, and *K. senegalensis* on *D. porcellus* leaves at different hours after treatment.

Plants	Solvent used	Concentration of extract (%)	Percent repellency after treatment			Mean repellency	Repellency class	Repellency index	Classification
			15 min	30 min	2 h				
B. ferruginea	Acetone	2.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.8 ± 21.7^{a}	0.0 ± 0.1^{abc}	Class 0	1.0 ± 0.0	Neutral
		5.0	7.1 ± 15.8^{a}	10.0 ± 7.0^{a}	0.0 ± 0.0^{a}	1.5 ± 6.9^{bcd}	Class I	0.9 ± 0.0	Repellent
		7.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 13.8^{a}	0.0 ± 0.0^{ab}	Class 0	1.1 ± 0.0	Attractant
	Propanol	2.5	0.0 ± 0.0^{a}	6.6 ± 20.1^{a}	10.6 ± 12.5^{a}	0.0 ± 7.2^{bc}	Class 0	1.0 ± 0.1	Neutral
		5.0	0.0 ± 0.0^{a}	25.0 ± 18.5^{a}	1.2 ± 15.1^{a}	3.4 ± 9.8^{bcd}	Class I	0.9 ± 0.0	Repellent
		7.5	2.1 ± 12.2^{a}	1.0 ± 18.9^{a}	17.5 ± 8.5^{a}	6.9 ± 7.8^{bcd}	Class I	0.9 ± 0.0	Repellent
	Ethanol	2.5	0.0 ± 0.0^{a}	0.5 ± 11.2^{a}	12.5 ± 14.9^{a}	2.8 ± 6.6^{abcd}	Class I	0.9 ± 0.0	Repellent
		5.0	0.0 ± 0.0^{a}	12.5 ± 8.5^{a}	2.5 ± 14.4^{a}	3.2 ± 8.1^{cd}	Class I	0.9 ± 0.0	Repellent
		7.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	19.7 ± 23.0^{a}	3.7 ± 9.9^{cd}	Class I	0.9 ± 0.0	Repellent
	Methanol	2.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.5 ± 11.8^{a}	0.0 ± 0.0^{ab}	Class 0	1.1 ± 0.0	Attractant
		5.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	15.0 ± 8.6^{a}	0.6 ± 7.9^{abc}	Class I	0.9 ± 0.0	Repellent
		7.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	13.4 ± 14.6^{a}	0.0 ± 3.4^{ab}	Class 0	1.0 ± 0.0	Neutral
	Distilled water	2.5	0.0 ± 0.0^{a}	18.1 ± 11.0^{a}	0.0 ± 0.0^{a}	0.0 ± 11.3^{cd}	Class 0	1.0 ± 0.1	Neutral
		5.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	9.3 ± 10.8^{a}	0.0 ± 4.0^{abc}	Class 0	1.0 ± 0.0	Neutral
		7.5	19.2 ± 19.5^{a}	25.0 ± 18.9^{a}	13.0 ± 19.3^{a}	19.0 ± 10.1^{cd}	Class I	0.8 ± 0.1	Repellent
B. sapida	Acetone	2.5	0.0 ± 0.0^{a}	0.0 ± 23.5^{a}	13.6 ± 22.6^{a}	0.3 ± 12.2^{bcd}	Class I	0.9 ± 0.1	Neutral
		5.0	0.0 ± 0.0^{a}	10.0 ± 9.1^{a}	49.7 ± 17.6^{a}	13.8 ± 12.4^{cd}	Class I	0.8 ± 0.1	Repellent
		7.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	Class 0	1.1 ± 0.0	Attractant
	Propanol	2.5	4.6 ± 13.0^{a}	31.1 ± 18.9^{a}	32.5 ± 4.7^{a}	22.7 ± 8.0^{d}	Class II	0.7 ± 0.0	Repellent
		5.0	0.0 ± 0.0^{a}	10.3 ± 16.0^{a}	4.3 ± 16.1^{a}	3.5 ± 9.1^{cd}	Class I	0.9 ± 0.0	Repellent
		7.5	6.3 ± 19.7^{a}	5.7 ± 10.0^{a}	15.8 ± 12.3^{a}	9.3 ± 7.7^{c}	Class I	0.9 ± 0.0	Repellent
	Ethanol	2.5	0.0 ± 0.0^{a}	3.1 ± 20.0^{a}	14.1 ± 6.7^{a}	2.8 ± 7.7^{abcd}	Class I	0.9 ± 0.0	Repellent
		5.0	13.9 ± 18.3^{a}	22.6 ± 18.7^{a}	17.5 ± 19.7^{a}	18.0 ± 9.9^{cd}	Class I	0.8 ± 0.0	Repellent
		7.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	2.5 ± 18.9^{a}	0.0 ± 0.0^{a}	Class 0	1.1 ± 0.1	Attractant
	Methanol	2.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	11.7 ± 10.4^{a}	0.0 ± 1.3^{abc}	Class 0	1.0 ± 0.0	Neutral
		5.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	Class 0	1.0 ± 0.0	Neutral
		7.5	0.5 ± 10.4^{a}	0.0 ± 0.0^{a}	9.0 ± 7.4^{a}	1.4 ± 4.3^{abc}	Class I	0.9 ± 0.0	Repellent
	Distilled water	2.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	18.5 ± 4.3^{a}	2.0 ± 5.1^{abcd}	Class I	0.9 ± 0.0	Repellent
		5.0	15.6 ± 15.7^{a}	9.7 ± 25.5^{a}	13.1 ± 12.5^{a}	12.8 ± 9.8^{cd}	Class I	0.8 ± 0.0	Repellent
		7.5	16.8 ± 27.8^{a}	16.9 ± 12.8^{a}	46.9 ± 13.6^{a}	26.9 ± 11.0^{d}	Class II	0.7 ± 0.1	Repellent

Table 4: Continued.

Plants	Solvent used	Concentration of extract (%)	Percent repellency after treatment 15 min	30 min	2 h	Mean repellency	Repellency class	Repellency index	Classification
		2.5	7.5 ± 9.4^{a}	14.4 ± 4.8^{a}	7.5 ± 12.5^{a}	9.8 ± 5.4^{cd}	Class I	0.9 ± 0.0	Repellent
	Acetone	5.0	0.0 ± 0.0^{a}	2.5 ± 21.4^{a}	0.0 ± 0.0^{a}	0.0 ± 3.1^{abc}	Class 0	1.0 ± 0.0	Neutral
		7.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	1.0 ± 9.7^{a}	0.0 ± 0.0^{a}	Class 0	1.1 ± 0.0	Attractant
		2.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	5.0 ± 11.9^{a}	0.0 ± 1.8^{abc}	Class 0	1.0 ± 0.0	Neutral
	Propanol	5.0	19.2 ± 10.0^{a}	22.3 ± 16.3^{a}	48.7 ± 10.8^{a}	30.0 ± 7.7^{d}	Class II	0.6 ± 0.0	Repellent
		7.5	2.5 ± 22.9^{a}	0.0 ± 0.0^{a}	17.9 ± 33.7^{a}	5.4 ± 14.3^{cd}	Class I	0.9 ± 0.1	Neutral
		2.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	Class 0	1.2 ± 0.1	Attractant
K. senegalensis	Ethanol	5.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	Class 0	1.2 ± 0.1	Attractant
		7.5	0.0 ± 0.0^{a}	15.0 ± 9.5^{a}	5.9 ± 12.4^{a}	0.4 ± 8.3^{abcd}	Class I	0.9 ± 0.0	Repellent
		2.5	3.9 ± 9.3^{a}	8.3 ± 6.8^{a}	6.7 ± 20.5^{a}	6.3 ± 7.1^{cd}	Class I	0.9 ± 0.0	Repellent
	Methanol	5.0	22.5 ± 7.5^{a}	13.1 ± 12.0^{a}	20.0 ± 14.1^{a}	18.5 ± 6.1^{cd}	Class I	0.8 ± 0.0	Repellent
		7.5	0.0 ± 0.0^{a}	1.3 ± 12.3^{a}	4.4 ± 14.8^{a}	0.0 ± 7.0^{c}	Class 0	1.0 ± 0.0	Neutral
		2.5	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	20.0 ± 16.8^{a}	1.4 ± 10.1^{abc}	Class I	0.9 ± 0.1	Neutral
	Distilled water	5.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	Class 0	1.0 ± 0.0	Neutral
		7.5	18.6 ± 9.2^{a}	17.5 ± 25.3^{a}	13.4 ± 20.4^{a}	16.5 ± 10.2^{cd}	Class I	0.8 ± 0.1	Repellent

Means within the same rows followed by the same letter are not significantly different ($p < 0.05$).

Table 5: Mortality rate of *D. porcellus* treated by topical application with acetone, propanol, ethanol, methanol, and aqueous extracts of *B. ferruginea*, *B. sapida*, and *K. senegalensis* and weight loss after 21 days (mean ± SE). Mortality rate was corrected using Abbott's formula (4).

Name of the plant	Solvent used	Concentration of extract (%)	Mortality rate (%) after different periods of exposures (days)				Weight loss (%)
			2	7	14	21	
B. ferruginea	Acetone	2.5	18.9 ± 3.1^{abcd}	51.3 ± 12.8^{de}	56.7 ± 13.2^{cd}	61.9 ± 12.7^{c}	1.2 ± 0.4^{ab}
		5.0	16.2 ± 8.1^{abcd}	40.5 ± 6.9^{cde}	48.6 ± 6.8^{bcd}	50.0 ± 5.6^{bc}	3.2 ± 0.6^{abcd}
		7.5	29.7 ± 3.1^{bcd}	70.2 ± 6.8^{d}	75.6 ± 9.2^{d}	76.4 ± 10.7^{c}	1.2 ± 0.0^{ab}
	Propanol	2.5	-5.4 ± 2.7^{ab}	16.2 ± 5.1^{abcde}	29.7 ± 6.9^{bcd}	38.2 ± 7.4^{bc}	1.3 ± 0.4^{abc}
		5.0	16.2 ± 9.2^{abcd}	32.4 ± 14.2^{bcde}	40.5 ± 10.3^{bcd}	38.2 ± 13.0^{bc}	3.4 ± 0.4^{abcd}
		7.5	27.0 ± 11.1^{abcd}	51.3 ± 6.9^{de}	62.1 ± 3.1^{cd}	61.7 ± 2.9^{c}	1.8 ± 0.5^{abcd}
	Ethanol	2.5	18.9 ± 11.2^{abcd}	40.5 ± 12.8^{cde}	64.8 ± 9.2^{cd}	64.7 ± 8.3^{c}	1.2 ± 0.5^{ab}
		5.0	2.7 ± 4.4^{abcd}	16.2 ± 2.7^{bcde}	29.7 ± 10.3^{bcd}	32.3 ± 15.4^{bc}	3.2 ± 0.6^{abcd}
		7.5	13.5 ± 11.6^{abcd}	27.0 ± 6.8^{bcde}	32.4 ± 5.1^{bcd}	41.1 ± 0.0^{bc}	1.6 ± 0.4^{abcd}
	Methanol	2.5	10.8 ± 8.1^{abcd}	27.0 ± 14.2^{bcde}	35.1 ± 10.8^{bcd}	29.4 ± 11.7^{bc}	2.4 ± 0.9^{abcd}
		5.0	10.8 ± 5.1^{abcd}	37.8 ± 9.2^{cde}	45.3 ± 10.3^{bcd}	35.2 ± 11.2^{bc}	3.0 ± 0.3^{abcd}
		7.5	13.5 ± 7.6^{abcd}	37.8 ± 11.9^{cde}	43.2 ± 9.2^{bcd}	41.1 ± 10.7^{bc}	1.0 ± 0.3^{a}
	Distilled water	2.5	2.7 ± 4.4^{abcd}	2.7 ± 4.4^{abcd}	5.4 ± 5.1^{ab}	8.8 ± 2.9^{bc}	3.1 ± 0.4^{abcd}
		5.0	2.7 ± 4.4^{abcd}	13.5 ± 4.4^{abcde}	18.9 ± 9.3^{bcd}	14.7 ± 8.8^{bc}	4.2 ± 0.7^{d}
		7.5	0.0 ± 2.7^{abcd}	16.2 ± 5.1^{abcde}	18.9 ± 3.1^{bcd}	11.7 ± 3.3^{bc}	3.9 ± 0.1^{bcd}
B. sapida	Acetone	2.5	13.5 ± 7.6^{abcd}	43.2 ± 19.4^{bcde}	51.3 ± 20.9^{bcd}	55.8 ± 18.2^{bc}	1.0 ± 0.3^{ab}
		5.0	13.5 ± 9.8^{abcd}	18.9 ± 11.2^{abcde}	32.4 ± 11.9^{bcd}	29.4 ± 10.7^{bc}	3.6 ± 0.5^{abcd}
		7.5	21.6 ± 2.7^{abcd}	56.7 ± 8.8^{de}	70.2 ± 9.2^{cd}	70.5 ± 7.5^{c}	1.6 ± 0.1^{abcd}
	Propanol	2.5	10.8 ± 6.8^{abcd}	32.4 ± 11.9^{bcde}	40.5 ± 13.6^{bcd}	32.2 ± 14.8^{bc}	1.3 ± 0.3^{abc}
		5.0	18.9 ± 9.3^{abcd}	27.0 ± 10.2^{bcde}	37.8 ± 6.8^{bcd}	35.2 ± 5.8^{bc}	3.8 ± 0.4^{abcd}
		7.5	32.4 ± 5.1^{bcd}	56.7 ± 15.9^{de}	72.9 ± 12.8^{cd}	79.4 ± 10.0^{c}	2.7 ± 0.6^{abcd}
	Ethanol	2.5	5.4 ± 2.7^{abcd}	21.6 ± 5.1^{bcde}	35.1 ± 6.2^{bcd}	48.6 ± 5.1^{bc}	1.1 ± 0.3^{ab}
		5.0	8.1 ± 3.1^{abcd}	27.0 ± 10.2^{bcde}	35.1 ± 12.4^{bcd}	38.2 ± 13.0^{bc}	3.2 ± 0.3^{cd}
		7.5	13.5 ± 4.4^{abcd}	32.4 ± 5.1^{cde}	48.6 ± 5.1^{bcd}	52.9 ± 10.7^{bc}	1.6 ± 0.3^{abcd}
	Methanol	2.5	10.8 ± 9.2^{abcd}	27.0 ± 5.1^{bcde}	43.2 ± 10.2^{bcd}	44.1 ± 14.7^{bc}	2.3 ± 0.7^{abcd}
		5.0	13.5 ± 7.8^{abcd}	35.1 ± 11.6^{cde}	43.2 ± 13.5^{bcd}	41.1 ± 12.7^{bc}	2.6 ± 0.6^{abcd}
		7.5	10.8 ± 11.1^{abcd}	51.3 ± 5.4^{de}	70.2 ± 9.2^{cd}	70.5 ± 7.5^{c}	2.0 ± 0.2^{abcd}
	Distilled water	2.5	-8.1 ± 0.0^{a}	-5.4 ± 2.7^{a}	-2.7 ± 3.1^{a}	-11.7 ± 3.3^{a}	2.2 ± 0.1^{abcd}
		5.0	5.4 ± 8.1^{abcd}	18.9 ± 11.2^{abcde}	27.0 ± 9.2^{bcd}	32.3 ± 10.0^{bc}	2.7 ± 0.4^{abcd}
		7.5	16.2 ± 14.2^{abcd}	29.7 ± 16.2^{abcde}	40.5 ± 18.9^{bcd}	47.0 ± 20.6^{bc}	1.7 ± 0.2^{abcd}

Table 5: Continued.

Name of the plant	Solvent used	Concentration of extract (%)	Mortality rate (%) after different periods of exposures (days)				Weight loss (%)
			2	7	14	21	
K. senegalensis	Acetone	2.5	67.5 ± 10.8^{d}	45.9 ± 7.6^{de}	18.9 ± 5.4^{bcd}	58.8 ± 14.0^{bc}	1.7 ± 0.7^{abcd}
		5.0	37.8 ± 9.2^{bcd}	43.2 ± 8.1^{cde}	51.3 ± 6.9^{cd}	55.8 ± 15.4^{bc}	3.8 ± 0.2^{abcd}
		7.5	18.9 ± 5.4^{abcd}	54.0 ± 8.1^{de}	64.8 ± 6.8^{cd}	70.5 ± 3.3^{c}	2.1 ± 0.7^{abcd}
	Propanol	2.5	54.0 ± 13.5^{cd}	24.3 ± 13.2^{abcde}	29.7 ± 6.9^{bcd}	27.0 ± 9.2^{bc}	1.9 ± 0.3^{abcd}
		5.0	16.2 ± 6.8^{abcd}	37.8 ± 14.2^{cde}	43.2 ± 11.9^{bcd}	38.2 ± 13.0^{bc}	3.9 ± 0.4^{abcd}
		7.5	21.6 ± 8.1^{abcd}	59.4 ± 11.9^{de}	70.2 ± 13.5^{cd}	67.6 ± 14.7^{c}	1.3 ± 0.2^{abc}
	Ethanol	2.5	10.8 ± 2.7^{abcd}	32.4 ± 8.1^{cde}	40.5 ± 6.9^{bcd}	35.2 ± 7.5^{bc}	1.9 ± 0.3^{abcd}
		5.0	18.9 ± 6.9^{abcd}	35.1 ± 7.6^{cde}	40.5 ± 5.4^{bcd}	41.1 ± 4.8^{bc}	4.1 ± 0.4^{abcd}
		7.5	-2.7 ± 7.4^{abc}	21.6 ± 2.7^{bcde}	32.4 ± 2.7^{bcd}	32.3 ± 5.6^{bc}	2.4 ± 0.5^{abcd}
	Methanol	2.5	10.8 ± 6.8^{abcd}	24.3 ± 15.9^{abcde}	32.4 ± 15.5^{bcd}	32.4 ± 18.8^{bc}	1.2 ± 0.4^{ab}
		5.0	16.2 ± 9.2^{abcd}	24.3 ± 11.6^{abcde}	29.7 ± 11.2^{bcd}	26.4 ± 13.0^{bc}	4.4 ± 0.4^{d}
		7.5	21.6 ± 9.2^{abcd}	32.4 ± 5.1^{cde}	51.3 ± 12.8^{bcd}	50.0 ± 16.8^{bc}	1.1 ± 0.4^{ab}
	Distilled water	2.5	-5.4 ± 2.7^{ab}	5.4 ± 13.5^{abc}	13.5 ± 11.6^{abc}	23.5 ± 5.8^{bc}	3.0 ± 1.0^{abcd}
		5.0	-2.7 ± 3.1^{abc}	-2.7 ± 3.1^{ab}	8.1 ± 5.4^{abcd}	5.8 ± 4.8^{b}	4.2 ± 0.3^{d}
		7.5	10.8 ± 5.1^{abcd}	21.6 ± 9.2^{bcde}	40.5 ± 18.4^{bcd}	41.1 ± 22.0^{bc}	2.4 ± 0.8^{abcd}
Control		0	7.5 ± 2.5	7.5 ± 2.5	7.5 ± 2.5	15.0 ± 6.4	4.7 ± 0.3^{d}

Means in a column followed by different letters are significantly different at $\alpha = 0.05$.

Table 6: Contact activity of acetone, propanol, ethanol, methanol, and aqueous extracts of *B. ferruginea*, *B. sapida*, and *K. senegalensis* determined by topical application to *D. porcellus*.

Plants	Solvent used	LC_{50}[a] (μL/insect)	95% confidence interval		Slope ± SE	Intercept ± SE	Chi square (df[b])	p value
			Lower	Upper				
B. ferruginea	Acetone	3.68	1.66	7.57	0.07 ± 0.02	−0.28 ± 0.15	36.32 (10)	0.000
	Propanol	6.77	5.62	9.43	0.14 ± 0.02	−1.01 ± 0.16	29.35 (10)	0.001
	Ethanol	1.53	1.16	4.48	−0.08 ± 0.02	0.13 ± 0.15	41.71 (10)	0.000
	Methanol	34.73	9.03	51.14	0.01 ± 0.02	−0.34 ± 0.15	37.97 (10)	0.000
	Distilled water	24.08	13.06	40.74	0.04 ± 0.03	−1.15 ± 0.18	16.30 (10)	0.091
B. sapida	Acetone	6.92	5.02	12.05	0.06 ± 0.02	−0.43 ± 0.15	89.18 (10)	0.000
	Propanol	5.84	4.59	9.64	0.14 ± 0.02	−0.82 ± 0.15	60.42 (10)	0.000
	Ethanol	11.97	7.96	38.25	0.05 ± 0.02	−0.59 ± 0.15	19.08 (10)	0.039
	Methanol	6.72	5.43	10.16	0.08 ± 0.02	−0.60 ± 0.15	42.48 (10)	0.000
	Distilled water	8.14	6.47	20.16	0.26 ± 0.03	−2.18 ± 0.21	75.11 (10)	0.000
K. senegalensis	Acetone	0.29	0.15	1.14	0.01 ± 0.02	−0.00 ± 0.15	37.37 (10)	0.000
	Propanol	6.04	4.93	8.03	0.10 ± 0.02	−0.61 ± 0.15	53.54 (10)	0.000
	Ethanol	3.84	1.38	6.56	0.04 ± 0.02	−0.17 ± 0.15	16.42 (10)	0.088
	Methanol	10.47	7.67	29.46	0.06 ± 0.02	−0.70 ± 0.15	75.42 (10)	0.000
	Distilled water	11.86	5.26	31.02	0.12 ± 0.03	−1.41 ± 0.18	69.57 (10)	0.000

[a]LC_{50}: lethal concentration 50. [b]df: degrees of freedom.

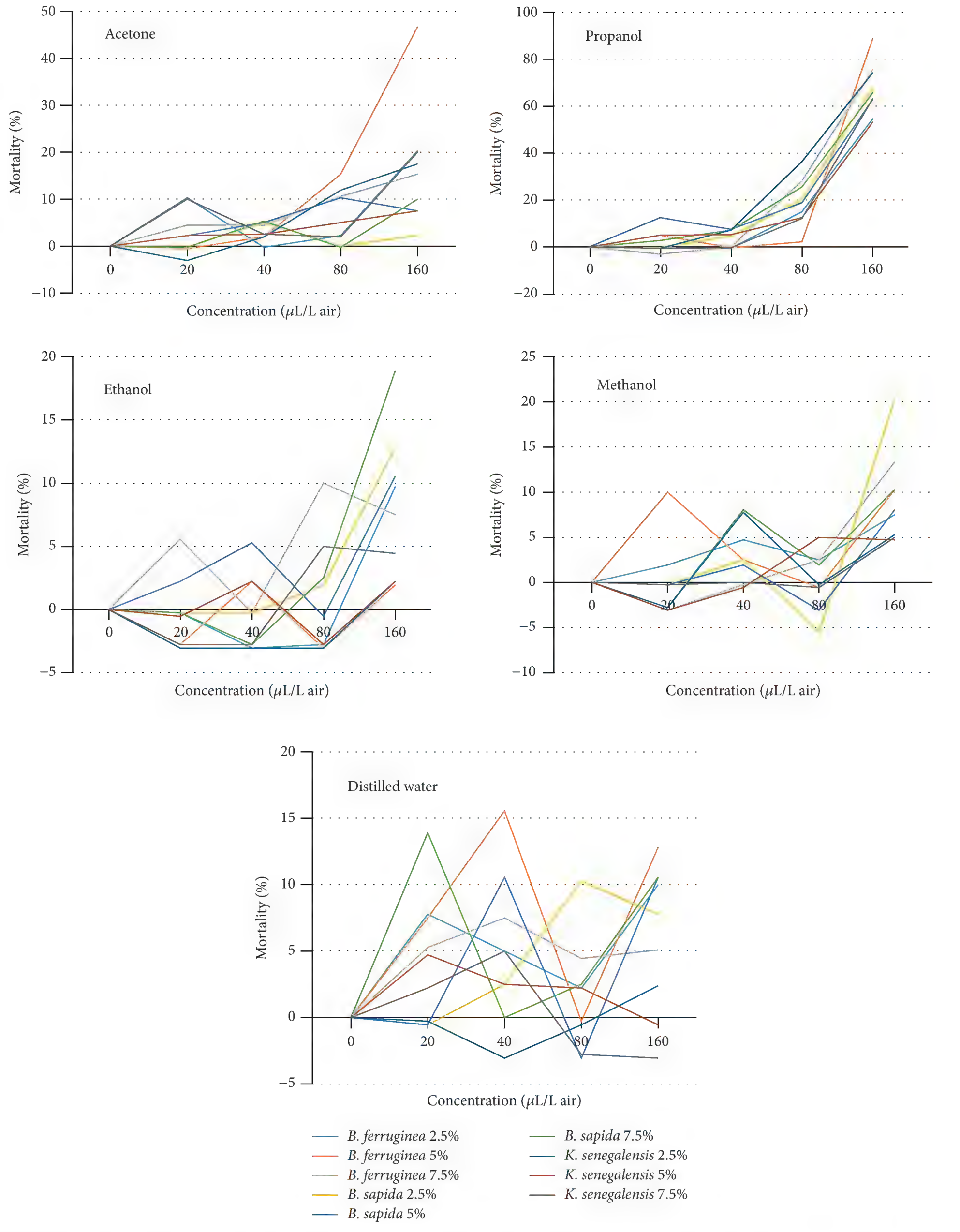

FIGURE 1: Percentage adult mortality of *D. porcellus* 24 hours after treatment with acetone, propanol, ethanol, methanol, and aqueous extracts of *B. ferruginea*, *B. sapida*, and *K. senegalensis*. Mortality rate was corrected using Abbott's formula (4).

Table 7: Fumigant toxicity of acetone, propanol, ethanol, methanol, and aqueous extracts of *B. ferruginea*, *B. sapida*, and *K. senegalensis* on adult of *D. porcellus* after 24 h exposure.

Plants	Solvent used	LC_{50}[a] (μL/L air)	95% confidence interval		Slope ± SE	Intercept ± SE	Chi square (df[b])	p value	TR_{50}[c]
			Lower	Upper					
B. ferruginea	Acetone	194.01	179.04	205.06	0.00 ± 0.03	−1.02 ± 0.18	100.19 (46)	0.000	—
	Propanol	18.68	10.84	42.65	0.04 ± 0.03	−0.86 ± 0.16	241 (46)	0.000	10.38
	Ethanol	28.45	13.36	91.98	0.06 ± 0.04	−1.77 ± 0.23	74.46 (46)	0.005	6.81
	Methanol	82.28	72.94	108.59	0.01 ± 0.03	−1.24 ± 0.20	43.83 (46)	0.563	2.35
	Distilled water	40.52	33.34	71.68	0.02 ± 0.03	−1.08 ± 0.19	62.51 (46)	0.053	4.78
B. sapida	Acetone	61.75	42.03	80.02	0.02 ± 0.04	−1.46 ± 0.21	50.88 (46)	0.287	3.14
	Propanol	55.90	49.26	68.80	0.01 ± 0.03	−0.61 ± 0.16	168.77 (46)	0.000	3.47
	Ethanol	180.11	165.74	200.55	0.00 ± 0.03	−1.36 ± 0.21	64.26 (46)	0.039	1.07
	Methanol	34.28	21.90	51.62	0.00 ± 0.04	−1.37 ± 0.21	66.53 (46)	0.025	5.65
	Distilled water	96.05	80.28	115.33	0.01 ± 0.03	−1.34 ± 0.20	82.63 (46)	0.001	2.01
K. senegalensis	Acetone	100.38	85.56	119.39	0.01 ± 0.03	−1.24 ± 0.19	70.63 (46)	0.011	1.93
	Propanol	5.40	5.29	6.28	0.06 ± 0.03	−0.32 ± 0.16	190.69 (46)	0.000	35.92
	Ethanol	39.24	22.53	70.12	0.04 ± 0.04	−1.87 ± 0.26	43.36 (46)	0.583	4.93
	Methanol	73.61	64.19	101.25	0.01 ± 0.04	−1.39 ± 0.22	40.41 (46)	0.705	2.63
	Distilled water	150.85	135.78	171.63	0.01 ± 0.04	−1.60 ± 0.24	27.26 (46)	0.987	1.28

[a]LC_{50}: lethal concentration 50. [b]df: degrees of freedom. [c]TR_{50}: toxicity ratio 50.

constituents such as terpenoids in leaves of *B. ferruginea* [35], *B. sapida* [36], and *K. senegalensis* [37], which are well-known repellents of phytophagous insects by acting in the vapor form on the olfactory receptors [38, 39]. The presence of some repellent components such as limonoids, which are highly oxygenated triterpenes, classed as tetranorterpenoides in leaves of *K. senegalensis* [40] could explain their higher repellent activities 24 h after treatment than synthetic insecticide Antouka and other plant powders. The protection of yam chips against insect damage provided by leaf powders of the three plants suggests that there may be an objective basis for their continuous use in traditional yam chips storage systems in Benin [2]. The results indicate that powders of *B. ferruginea*, *B. sapida,* and *K. senegalensis* leaves could be a source of novel repellent against *D. porcellus*. However, there is a need to increase the efficacy of such natural products by developing methods such as mixing with some fixative materials for long lasting efficacy [40].

The result of the study further showed that mortality of *D. porcellus* caused by the various concentrations of the three leaf powders used was higher in comparison with those observed in the negative control. This indicated that the leaf powders were poisonous to adult *D. porcellus* and could serve as a bioinsecticide. The insect mortality may be due to blocking of spiracles of the insect by dust particles and death caused by asphyxia [41]. Plant product may also penetrate the insect body via the respiratory system [42]. Further, Sousa et al. [43] reported that the plant powders caused dehydration to insects by erosion of cuticle layer and their death occurred subsequently. Insecticidal effect of the three botanicals may also be due to its active components. Insecticide activity of *B. sapida* is primarily due to saponins and tannins [36, 44] and might be the cause of the insecticidal activity in this study. *K. senegalensis* leaf powder possesses limonoids which have a wide range of biological activities, including insecticidal, insect antifeedant, and growth regulating activity on insect [14]. Researchers found that *B. ferruginea* has insecticidal properties [45]. However, further investigations are required to determine the efficacy and the active ingredients present in the three plants as well as synthesize them and make scientific formulations for effective use in controlling *D. porcellus*.

Adding powders of *B. ferruginea*, *B. sapida,* and *K. senegalensis* leaves to the yam chips not only increases the mortality of *D. porcellus* but affects the weight loss and the median development time of *D. porcellus* F1 progeny. Similar findings were reported by Mukanga et al. [46] who show that leaf powders of five botanicals (eucalyptus, guava, neem, *Tephrosia*, and water hyacinth) reduce weight loss and clearly suppressed the emergence of *Prostephanus truncatus* populations in dried cassava chips. Based on previous studies, much of the antifeeding of test botanicals could be attributed to their bioactive principles [14, 36, 44, 45]. The inhibition of reproduction rate of *D. porcellus* could be due to the changes in physiology and behaviour in the insect adults due to contact with botanicals that may deter their egg laying capacity [42]. However, the efficacy of the plant products in significantly suppressing emergence has largely been attributed to ovicidal properties, which prevent eggs from hatching into larvae [47] and/or larvicidal activity which caused the larvae from maturing to adult. The good inhibitory effects of powder *B. ferruginea* leaves on the reproductive cycle in which the F_1 progeny was reduced by 50% give a glimmer of hope for use as yam chips protectants against *D. porcellus*.

The results regarding the evaluation of repellent potential of different extracts revealed that the plant crude extracts tested showed a lower repellency than botanical powders. There was considerable variation in the repellent action of the various botanicals extracts. This variation could be explained by the fact that the type of solvent selected affects extract efficacy due to different phytochemicals of varying volatility being present in the final extraction [48]. Among all the plant species, concentrations, and solvents tested, propanol extract of *K. senegalensis* at 5% was found to be the one with the highest repellent effect on *D. porcellus* with percentage repellency of 30%. Strangely, in our laboratory experiments, we found an attractive effect of acetone extract of *B. ferruginea*, *B. sapida,* and *K. senegalensis* at 7.5%, the methanol extract of *B. ferruginea* at 2.5%, and the ethanol extract of *B. sapida* at 7.5% and *K. senegalensis* at 2.5 and 5% on the beetle *D. porcellus* while the other plant extracts were repellent or neutral. The reason for this kind of both repellent and attractant at different concentration is unknown. Similarly, Pugazhvendan et al. [49] found that hexane, chloroform, and ethyl acetate extracts of *Artemisia vulgaris*, *Sphaeranthus indicus*, *Tephrosia purpurea*, and *Prosopis juliflora* can be repellent or attractant on *Tribolium castaneum* at different concentrations. Our findings suggest that there may be different compounds in different solvent extracts possessing different bioactivities. The biological activities of the three tested plants merit further investigation to determine the active ingredients responsible for their repellent or attractant properties.

The results of topical application of crude extracts of *B. ferruginea*, *B. sapida,* and *K. senegalensis* suggest that the leaves of these plants have the greatest potential for insecticidal activity against *D. porcellus*. Earlier studies confirmed the insecticidal effects of these plants species: *B. ferruginea* [45]; *B. sapida* [15, 36, 50–53]; and *K. senegalensis* [54–59]. Acetone extract of *K. senegalensis* showed the lowest LC_{50} and the biggest toxicity ratio, consequently the most toxic on *D. porcellus*. Some studies proved efficacy of *K. senegalensis* for controlling stored products insects such as *Trogoderma granarium* [59], *Callosobruchus maculatus* [54, 55], and *Tribolium confusum* [58]. The phytoconstituents found in the leaf extract of *K. senegalensis* include tannins, saponins, flavonoids, steroids, and alkaloids [37]. The presence of these organic compounds in the plant extract may have been responsible for the mortality of *D. porcellus*. Based on the 24 h LC_{50} values obtained, the acetone extract was more potent than the other extracts used in this study, suggesting that the organic solvent enhanced the extraction/release of the active principle(s). Results indicate that the extracts of the different plants can be incorporated as biopesticides in pest management programs against *D. porcellus*.

Fumigation studies of different plants extracts at different concentrations showed variable toxicity to adults of *D. porcellus*, depending on plant species and solvents. Propanol

extract of *B. ferruginea* at 5% exhibited significant fumigant toxicity to *D. porcellus*. This indicated that propanol extract of *B. ferruginea* at 5% might be useful for managing *D. porcellus* in enclosed spaces such as storage bins, glasshouses, or buildings because of their fumigant action. The fumigant toxicity of propanol extract of *B. ferruginea* at 5% as well as the other plant extracts could be attributed to major constituents such as monoterpenoids [35]; due to their high volatility, they have fumigant and gaseous action which might be of importance for stored-product insects [60]. Monoterpenoids are typically volatile and rather lipophilic compounds, which can rapidly penetrate into insects and interfere with their physiological functions [61]. The results of these fumigation studies indicate that effective extracts of *B. ferruginea*, *B. sapida,* and *K. senegalensis* fumigation treatments against *D. porcellus* can also be potentially applicable in the integrated pest management of this pest.

5. Conclusion

Results from the current study confirmed the importance of the use of *B. ferruginea*, *B. sapida,* and *K. senegalensis* by farmers in Benin to protect stored yam chips of *D. porcellus*. It is evident from the above results that the tested botanical powders are potential protectants (repellents and antifeedants) of stored durable yam chips against *D. porcellus*. The findings of this research have also shown insecticidal effects (contact and fumigation) of extracts of the three medicinal plants. These results indicate that both plant powders and extracts have potential for yam chips protection. They can be used as an alternative to synthetic insecticides. However, the identification and isolation of bioactive compounds from the powders and extracts of these three medicinal plants must be done as key issue for further study.

Conflicts of Interest

The authors have not declared any conflicts of interest.

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Research Article

The First Workers of the Ant *Camponotus obscuripes* Are a Different Allometric Morph with Relatively Long Antennae to Communicate with Other Larger Colony Members

Saori Watanabe,[1] Mamoru Terayama,[2] Ryota Kawauchiya,[3] Natsuki Ogusu,[1] Yusuke Fujita,[1] Syunta Mikami,[1] Yuuka Murakami,[4] and Eisuke Hasegawa[1]

[1]*Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan*
[2]*Laboratory of Applied Entomology, Department of Agricultural and Environmental Biology, The University of Tokyo, Tokyo 113-8657, Japan*
[3]*Institute of Entomology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan*
[4]*Department of Neuropharmacology, Graduate School of Medicine, Hokkaido University, Sapporo 060-8638, Japan*

Correspondence should be addressed to Eisuke Hasegawa; ehase@res.agr.hokudai.ac.jp

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The first workers produced by an ant queen with a claustral founding mode are much smaller than the workers after the second generation and are thus called "nanitics." These nanitics shoulder the initial fate of the colony and thus may be different morphometric morph from the other workers in mature colony to optimize the survival of their own colony. We report here that, in the ant *Camponotus obscuripes* Mayr, the allometric rules of the nanitics are different from those of other workers in mature colonies, suggesting that the nanitics constitute an independent caste as with soldiers or queens in other species. In addition, the antennae of the nanitics show the minimum absolute length-difference with the mother queen compared to the other traits measured. This result suggests that this small size difference enables *C. obscuripes* nanitics to communicate with the other members of the colony. Our results indicate that polymorphic societies affect the growth rules of workers.

1. Introduction

Many ant species show the claustral nest-founding mode, in which a dealated queen produces a few 1st workers using only the reserved nutrition in her body [1–3]. Eclosed 1st workers are much smaller than the workers after the 2nd generation and are thus called "nanitics" [2]. The nanitics are special workers that shoulder the fate of the colony. Since the amount of resources that can be invested into the production of the nanitics is limited to the stored nutrients in the queen's body, a trade-off between size and numbers is expected to occur among the nanitics produced by a queen [4]. In fact, this trade-off has been detected in the nanitics of the monogynous ant *Camponotus japonicus* Mayr, 1866 [5]. A previous study showed that there is a negative correlation between the number and the size of the nanitics when a queen's investment into them was controlled statistically [5]. Therefore, the nanitics are assumed to be under strict constraints in terms of their development to maintain the optimal relationship between size and number. This suggests that the nanitics may have different growth rules relating morphological traits to body size compared with other workers in mature colonies.

From the analysis of the slope and the intercept of this equation, we can determine the growth rules of the traits. In some cases, the allometric rule of a trait changes with body size, namely, a morphological polymorphism. For example, males of a stag beetle, *Prosopocoilus inclinatus* (Motschulsky, 1857), show a dimorphism in the mandible size to the body

size [6]. The male mandible of stag beetles is a special organ used to fight with other males, and the two morphs adopt different reproductive strategies [7, 8]. Thus, the growth rules are affected by the ecology of the focal organism.

In ants, worker polymorphisms are classified into five types, that is, monomorphism, monophasic allometry, diphasic allometry, triphasic allometry, and complete dimorphism [9]. A species showing distinctive polymorphisms has more than one worker-morph, each of which has a different allometric rule for some traits, for example, the small normal workers and the soldiers with a huge head in species of the ant genus *Pheidole* [complete dimorphism [10]]. In this distinct polymorphism, each caste has a different allometric rule for some trait (e.g., the head of workers in *Atta* spp.). In this type, the allometric line has one (or more) inflection point(s), meaning that there is more than one morph, each of which has a different allometric rule [9, 11]. Another type is monophasic allometry, in which the size of workers varies considerably within a colony, but the allometric rule is represented by a simple linear regression, with *Camponotus obscuripes* Mayr 1879 belonging to this type [12].

In a monophasic allometric ant, *Solenopsis invicta* Buren, 1972, allometric rules have been shown to differ depending on the colony size or the social form (monogyny or polygyny) [13]. In *S. invicta*, the allometric equation of the ratio of the antennae/body size to the body length has a negative slope [13]. In our interpretation, this result suggests that the antennae of *S. invicta* are under a constraint in terms of their absolute length, likely because the ants communicate with other colony members by physical contacts with the antennae, and thus, communication may occur only between workers with antennae of a similar length.

We generalized two hypotheses to be tested from this consideration; (1) antennae are the smallest divergent trait between small and large individuals in an ant species, and (2) nanitics may have different allometric rules for several traits (especially for antennae) comparing with workers in mature colonies, meaning that nanitics are a distinct morphological caste as with soldiers in the complete dimorphic species.

In this study, we tested these hypotheses using a monophasic allometric ant, *C. obscuripes*. We compared the allometric rules for several traits of the nanitics and workers in mature colonies. In addition, we checked the degree of the difference in absolute length for examined traits between the nanitics and their mother queens. Based on the results, we tested the above hypotheses and will discuss how the social lives of ants affect the morphological growth rules of workers belonging to different generations.

2. Materials and Methods

2.1. Study Organisms. We examined a monophasic allometric ant, *Camponotus obscuripes*. Eighteen incipient colonies were obtained (see below). Each individual was separated into the head, thorax, right-side legs, and abdomen. These body parts were fixed to a glass slide using an adhesive agent and were dried at 60°C for 3 days before the measurements. All the measured traits are exoskeleton parts, and thus, the drying did not shrink the sizes of the traits.

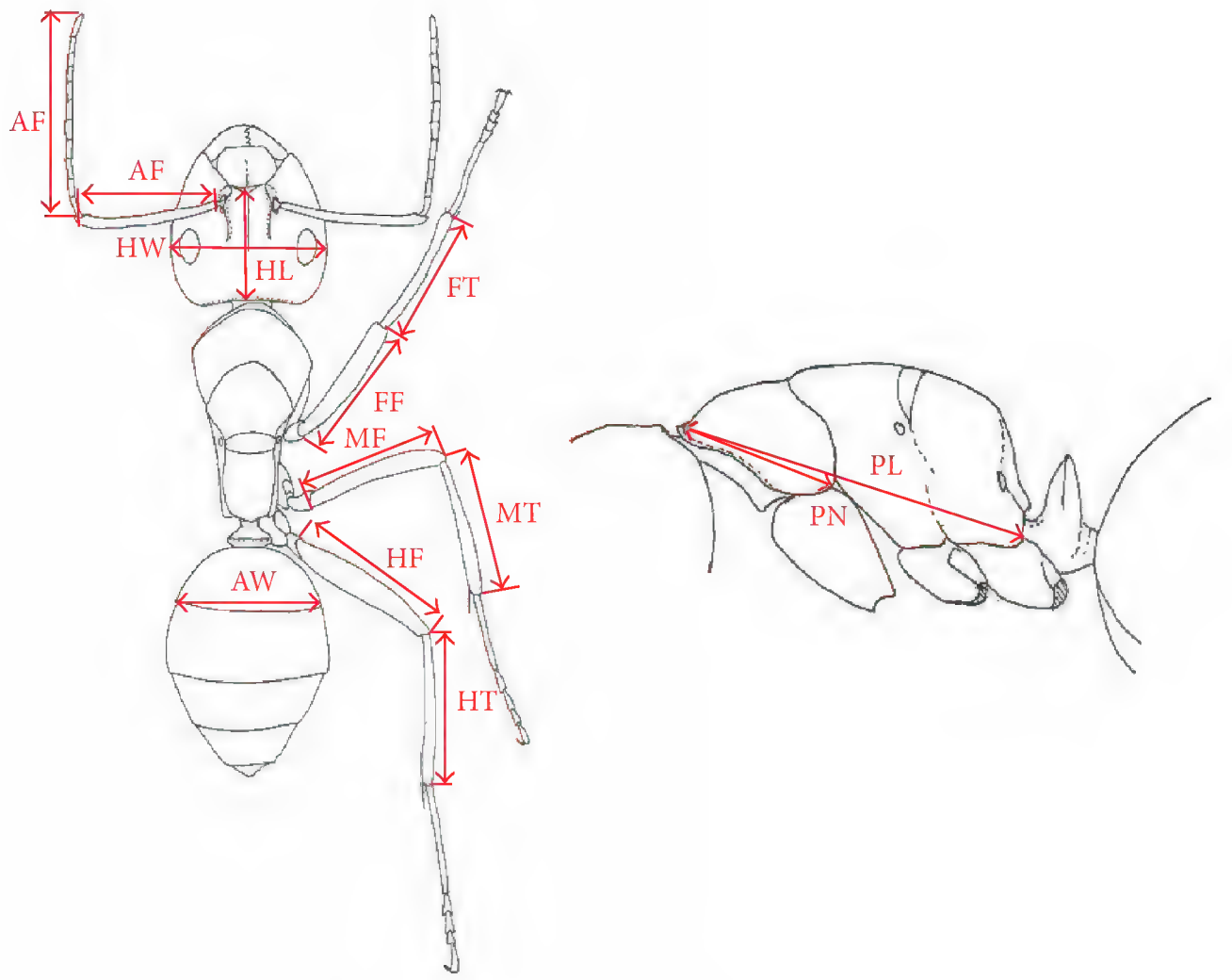
The allometric rules of the workers in mature colonies were examined using a sample from a mature colony from Niigata Pref., Japan. Thirty-three workers that covered the full size range of the colony were prepared as described in the previous paragraph. All the individual body parts were photographed with a scale bar (10 mm) using a digital camera (WRAYCAM-NF300, WRAYMER, Osaka, Japan) attached to a binocular microscope (SZH, OLYMPUS, Tokyo, Japan).

In autumn 2016, eighteen incipient colonies (including a queen and a few nanitics) of *C. obscuripes* were bought from an online insect shop (the Ant-Room; http://www.antroom.jp/about.php). All the queens were collected in the summer of 2016 in Hachioji, Tokyo, Japan.

2.2. Morphological Measurements. We measured several morphological traits of all the individuals (see Figure 1; head width (HW), head length (HL), thorax length (TL), pronotum length (PN), length of right forefemur (FF), foretibia (FT), midfemur (MF), midtibia (MT), hindfemur (HF), hindtibia (HT), width of posterior end of 1st segment of abdomen (AW), and lengths of the left antennal scape (AS) and the antennal funiculus (AF)) to 0.001 mm using a freeware (ImageJ, ver. 1.45) on the digital photographs. For the mature colony, we selected 33 workers that covered the full size range of the sample. For the incipient colonies, we measured 18 queens and a total of 78 nanitics (2 to 7 nanitics per queen).

First, we conducted a principal component analysis of the workers in the mature colony to determine the index of body size. As the antennae are focal objectives, we removed AS and AF from this analysis. Relationships of growth among organs are represented by the allometric rules between two traits [14]. In an allometric analysis, a trait is selected as the index of body size (for the selection method, see [15], pp 203–205), and the regression of other traits on the body size is calculated using the log-transformed data. The trait that is the most isometric with the principal component 1 is used as the index of body size (see [15], pp 203–205). AW was selected as the index of body size. Then, we calculated allometric equations for the remaining traits based on AW for the nanitics, the workers in the mature colonies, and the queens using log-transformed data. Then, linear regression equations were calculated for each class. The slopes and the intercepts of the two allometric equations for each trait pair were compared within the 3 classes using ANCOVA.

Second, we compared the absolute sizes of each trait between the queen and her nanitics as (the worker's length)/(the queen's length) to determine which trait shows the lowest size difference between the queens and the nanitics. Using the 76 data points for each trait, we compared each pair of traits for significant differences between the two traits using the Wilcoxon matched pairs test. The significance level was corrected using the Bonferroni correction for multiple comparisons. In addition, we calculated the same ratios for the workers in the mature colony between the largest and smallest workers (in AW).



AF: antennal funiculus
AS: antennal scape
HW: head width
HL: head length
TL: thorax length
PN: pronotum length
FF: right forefemur
FT: right foretibia
MF: right midfemur
MT: right midtibia
HF: right hindfemur
HT: right hind tibia

Figure 1: The traits measured.

3. Results

First, there is no significant correlation between the sizes of the same trait of the queens and her nanitics for all the measured traits (Pearson's correlation coefficient; for all the traits, $p > 0.05$), meaning that we can treat each nanitic's data as an independent data point because these results showed that there is no phenotypic correlation between the mother queen and her nanitics. Probably, the expected genetic correlation has been covered by differences in nutritional condition among queens because sizes of nanitics have shown to be affected by the initial amount of resources stored in their queen [5]. Thus, we combined all the data for the incipient colonies for the statistical analyses.

Figure 2 shows the allometric relationships for each trait in each class (nanitics, workers in a mature colony, and queens). In several traits, there is a difference in the allometric slopes between the nanitics and the other workers (i.e., HW, HL, TL, FF, FT, and MF); the differences are significant after the Bonferroni correction for multiple comparisons (see Figure 2). But note that, for AF, there is no difference in the slopes among the three classes. For the workers in the mature colonies, only the slope of HW is significantly steeper than 1.0, that is, the monophasic allometry in them. The slopes of MT, HF, and HT do not differ between the nanitics and the other workers. In conclusion, the nanitic is a distinct caste that follows different growth rules from those of the other workers for several traits.

Figure 3 shows the proportions of a dwarf trait to the same trait of their mother queen (Figure 3(a)) and those between the largest and smallest workers in the mature colony (Figure 3(b)). In the former, AS and AF are the traits that show the first and second smallest differences in absolute length compared to the queens. These differences are still significant after the Bonferroni correction for multiple comparisons (Figure 3(a)). As a result, the two parts of antennae (AF and AS) have the lowest differences in length compared to the queen. Although we could not conduct a statistical test, this trend is the same for the workers in the mature colony (Figure 3(b)).

4. Discussion

Our first finding is that the nanitics of *C. obscuripes* make up a distinct morphological caste that has different allometric rules for several traits from those of the other workers in the mature colony. The existence of nanitics in claustral founding ants has been thought to be a cause of their success in the ecosystem [3]. Because of the limited amount of resources needed to produce nanitics, a trade-off between size and

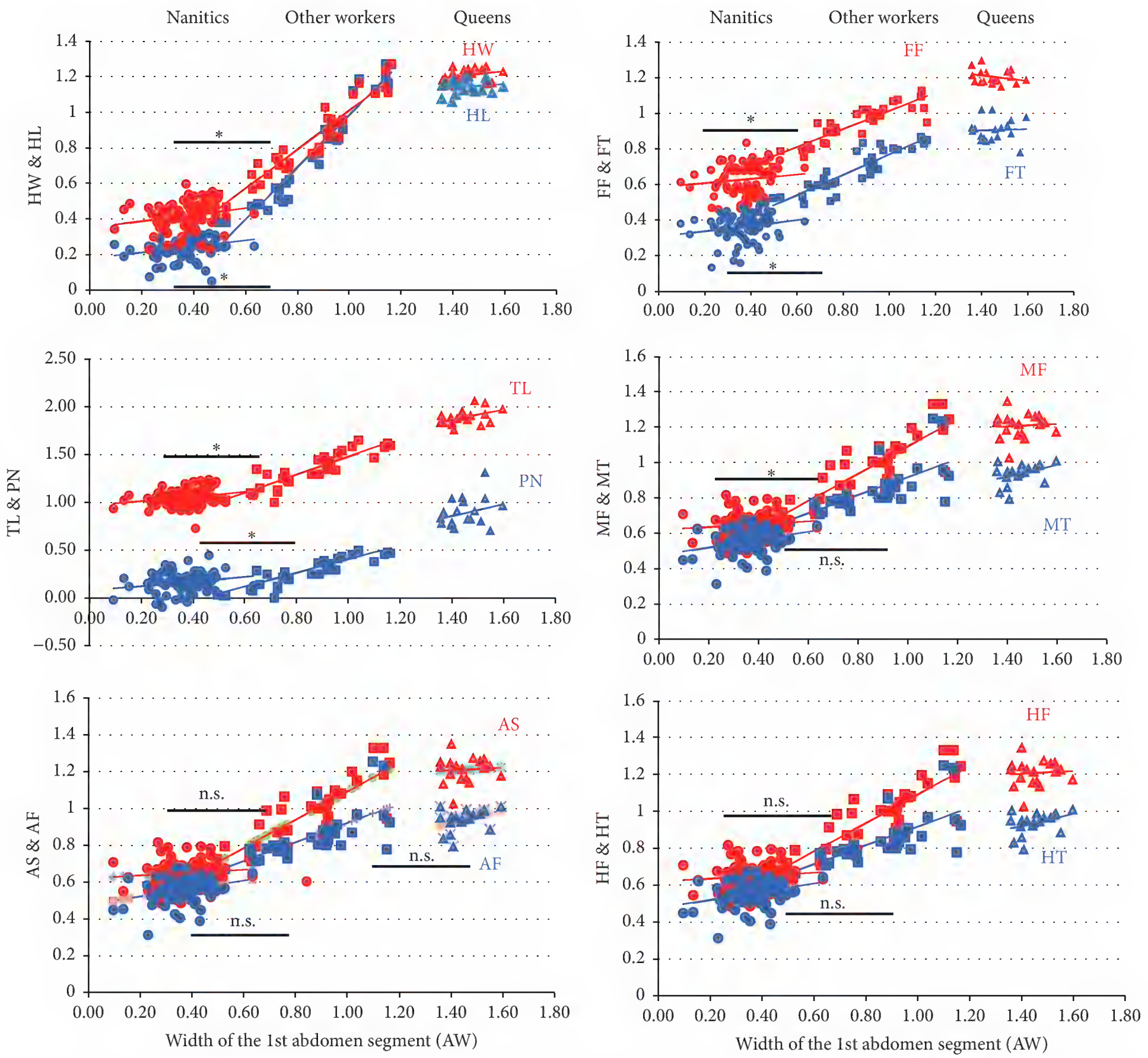


Figure 2: Allometric pattern in each trait for each class (nanitics (left circle), other workers (central squares), and queens (right triangles)). Significant differences among the classes (the compared class is shown with horizontal black bars) were tested by ANCOVA. The stars (∗) represent a statistically significant difference in the slopes after Bonferroni correction for multiple comparisons among the classes (the significance level is $p = 0.0042$ in this case). The fact that the queens are a distinct caste is apparent; the queen class was removed from the calculations excluding the analysis for AF, in which the allometric slope for the queens is not different from those of the other two classes.

number is expected [4], and a previous study has supported this hypothesis [5]. Because nanitics shoulder the initial fate of the colony, their body shape should be strongly constrained to the optimal growth rule to maximize colony survival. Thus, it is not surprising that the nanitics have distinct allometric rules from those of other workers. Our results demonstrate this, and we conclude that nanitics of *C. obscuripes* constitute a distinct caste from the other workers, like soldiers in species with the complete dimorphism.

A distinctive allometric pattern among the 3 classes (nanitics, other workers, and queens) has led us to a conclusion that *C. obscuripes* is a species showing the diphasic allometry rather than the monophasic allometry as believed previously [9]. The workers in a mature colony have a simple linear regression slope in each trait (Figure 2) suggesting they show the monophasic allometry, but the nanitics show significantly different slopes for several traits (i.e., HW, HL, TL, PN, FF, FT, and MF) from the workers in the mature colony (Figure 2). These results suggest that other ant species showing the monophasic allometry may be substantially diphasic as with *C. obscuripes*. Examinations of allometric rules in nanitics are needed to confirm this hypothesis.

The diphasic allometry typically found in HW, that is, the allometric slope, is moderate in the nanitics and the queens

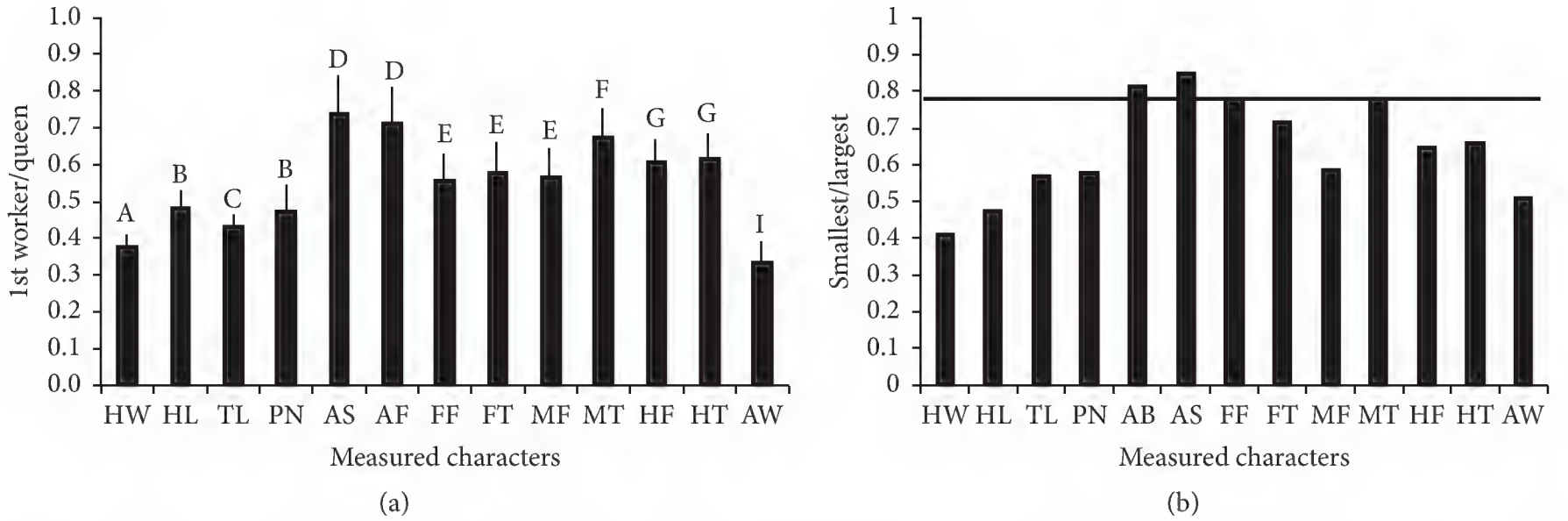


Figure 3: The ratios of a dwarf's trait length to her mother queen's length of the same trait. (a) The ratio of dwarf to queen traits. The different letters on the whiskers indicate statistically significant differences after Bonferroni correction for multiple comparisons. (b) The ratio of the smallest workers to the largest ones in the mature colony. AS and AF show the first and second smallest differences in these lengths. The black horizontal bar in (b) shows the value of the trait that shows the third smallest difference (FF).

but is steep in the other workers (see Figure 2). This pattern is found in several other traits. For example, TL, PN, FF, FT, and MF show similar patterns with HW. It is noteworthy that these traits are thought to support directly the weight of the head. A previous study has shown that workers of ants have relatively large pronotum in which there are many neck muscles that support head weight [16]. The allometric slope of HW (1.472) is significantly steeper than 1 (ANCOVA, $t = 11.095$, df $= 32$, $p = 1.679e - 12$), indicating that HW increases disproportionately with body size. As HW becomes large, the head weight will increase to the third power of the increase in HW. Thus, supportive traits of the head weight would show a similar allometric pattern as that of HW. In male stag beetles with exaggerated mandibles as weapons, supportive traits of the weapons (head or prothorax) are also enlarged [17, 18].

It is unknown why the worker's head disproportionally enlarges with body size in *C. obscuripes*. The enlarged head of the large workers in *C. obscuripes* may function as a food crusher as in soldiers of *Pheidole* spp. [10]. Nevertheless, an enlarged head would need robust supportive traits to be fully functional. Therefore, several such traits (TL, PN, FF, FT, and MF) may show a similar allometric growth pattern as that of HW.

The hind leg does not show the above pattern and instead seems to be monomorphic with AW. Hind legs may be used mainly to support the weight of the abdomen and thus increase constantly with abdomen size irrespective of the worker size. This hypothesis seems to be supported by the observed allometric patterns found in HF and HT.

The second finding is the smallest difference in the absolute length of antennae between large and small individuals. Figure 3(a) shows the proportions of a nanitic's trait size to her mother's. The AS and AF of the nanitics are significantly closer to those of the queen compared with the other traits after a Bonferroni correction for multiple comparisons (Figure 2). Ants communicate with nest mates using their antennae. In particular, the antennal funiculus seems to be important because it contains many sensory receptors [19]. If there is a large difference in the AF between individuals, efficient communications may be difficult.

This constraint comes from sociality. As colony members must communicate with nest mates to maintain colony function, the length of the antennae cannot differ substantially among the nest members. Solitary insects would be free from this constraint and, thus, the observed feature of the antennae may be restricted in social insects. However, we cannot test this hypothesis using ants because there is no ant species without sociality, excluding the social parasites (the antennae of a social parasite would become of a similar length to that of the host species due to the requirement for communicating with host individuals). However, in social wasps belonging to the Polistinae, the 1st workers are smaller than those after the second generation [20]. If social Polistinae wasps showed the same trend in antennal length as that of *C. obscuripes,* we could test the size constraint hypothesis by comparing the difference in antennal length to the body size between solitary and social wasps. The smallest difference in antennal length between large and small individuals would be a trait that has evolved after acquiring sociality. This hypothesis is worth being tested in future studies. In conclusion, the acquisition of sociality differently affects the growth rules of different castes in a colony to optimize colony efficiency for survival.

Disclosure

The English in this document has been checked by a professional editor who is a native speaker of English. The certificate is available by Eisuke Hasegawa. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors declare no financial conflicts of interest.

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Research Article

An Insight in the Reproductive Biology of *Therophilus javanus* (Hymenoptera, Braconidae, and Agathidinae), a Potential Biological Control Agent against the Legume Pod Borer (Lepidoptera, Crambidae)

Djibril Aboubakar Souna,[1,2,3] Aimé Bokonon-Ganta,[3] Marc Ravallec,[1] Antonino Cusumano,[4] Barry Robert Pittendrigh,[5] Anne-Nathalie Volkoff,[1] and Manuele Tamò[2]

[1]*UMR DGIMI 1333 INRA, UM, Case Courrier 101, Place Eugène Bataillon, 34 095 Montpellier, France*
[2]*International Institute of Tropical Agriculture, Benin Research Station (IITA-Benin), 08 BP 0932 Cotonou, Benin*
[3]*Faculté des Sciences Agronomiques (FSA), Université d'Abomey Calavi, 01 BP 526 Cotonou, Benin*
[4]*Department of Plant Sciences, Wageningen University & Research, Wageningen, Netherlands*
[5]*Department of Entomology, Michigan State University (MSU), East Lansing, MI, USA*

Correspondence should be addressed to Anne-Nathalie Volkoff; anne-nathalie.volkoff@inra.fr
and Manuele Tamò; m.tamo@cgiar.org

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Therophilus javanus is a koinobiont, solitary larval endoparasitoid currently being considered as a biological control agent against the pod borer *Maruca vitrata*, a devastating cowpea pest causing 20–80% crop losses in West Africa. We investigated ovary morphology and anatomy, oogenesis, potential fecundity, and egg load in *T. javanus*, as well as the effect of factors such as age of the female and parasitoid/host size at oviposition on egg load. The number of ovarioles was found to be variable and significantly influenced by the age/size of the *M. vitrata* caterpillar when parasitized. Egg load also was strongly influenced by both the instar of *M. vitrata* caterpillar at the moment of parasitism and wasp age. The practical implications of these findings for improving mass rearing of the parasitoid toward successful biological control of *M. vitrata* are discussed.

1. Introduction

The legume pod borer *Maruca vitrata* (Fabricius) (syn. *M. testulalis*) (Lepidoptera: Crambidae) is an important pest of cowpea, *Vigna unguiculata* L. Walp (Fabales: Fabaceae), a widely cultivated legume crop in Sub-Saharan Africa, and can cause yield losses in the range of 20–80% [1]. According to taxonomic and population genetic studies, the putative area of origin of this pest is assigned to South Asia [2]. *Therophilus javanus* (Bhat & Gupta, 1977) (Hymenoptera: Braconidae) is an endoparasitoid that develops inside *M. vitrata* during the early larval stages. High parasitism rates of *T. javanus* on *M. vitrata* caterpillars have been reported in soybean and yard-long beans fields in tropical Asia, Lao PDR, Vietnam, and Southern Taiwan [3, 4]. *T. javanus* thus seems an excellent candidate for use as a biological control agent against *M. vitrata* in West Africa. However, the development of biological control relying on *T. javanus* releases requires a thorough knowledge of its basic biology, which has not been investigated yet.

Therophilus javanus belongs to the Agathidinae subfamily of Braconidae, which includes an estimated two to three thousand species worldwide with a higher number of genera in tropical than in temperate regions [5]. Some species have been employed as biological control agents against various insect pests [6, 7]. Although Agathidinae have been

studied for taxonomic or phylogeny purposes, the biology of members of this subfamily remains largely unknown. A few biological and quite ancient studies have been conducted on Agathidinae oviposition and larval development [8–11]. Most studied Agathidinae species oviposit into special organs (nerve ganglia) [12], but some, including *T. javanus*, place their eggs directly into the host hemocoel [8–10]. Apart from a few studies, that is, the number of eggs laid by *Bracon vulgaris* (Hymenoptera: Braconidae) [11], and the number of offspring produced by *Agathis gibbosa* (Hymenoptera: Braconidae) [9], there is a dearth of data concerning Agathidinae reproductive biology.

Fecundity is one of the proxies used by biologists to investigate the individual fitness [13] and may greatly vary depending on the species and its life cycle. It can also be affected by a series of abiotic (e.g., temperature) and biotic (e.g., wasp nutritional status, mating status, and age) parameters. Fecundity has been shown to correlate positively with the number of ovarioles, that is, the egg-producing components of the ovary [14]. The number of ovarioles is commonly species-specific, and there is great variation across insects, ranging from less than five per ovary in some flies to hundreds per ovary in some grasshoppers [15]. As such, ovariole number is relatively stable for a given species but can vary due to different environmental or nutritional conditions [16]. In some parasitoid species, adults emerge with their full load of mature eggs (termed "proovigenic") while other species mature eggs during their adult life (termed "synovigenic") [17]. For the latter, production of the first eggs relates to the amount of nutritional resources stored during larval stages [18, 19].

In the present work, we investigated *T. javanus* reproductive biology, ovariole number, egg development, and the potential fecundity, as well as how egg loads vary depending on the wasp female age and how they are affected by parameters such as the nutritional quality provided by the lepidopteran host (i.e., caterpillar instar).

2. Materials and Methods

2.1. Insect Rearing. Therophilus javanus was provided by The World Vegetable Center (AVRDC), Taiwan, Republic of China, and reared for several generations under confined conditions at IITA, Benin research station. The pod borer *M. vitrata* colony was established from feral populations collected from both cowpea fields and alternative host plants surrounding the IITA-Benin station. Insect colonies were reared under laboratory conditions, with 12 : 12 L : D photoperiod 26°C ± 1.1°C average temperature and 76% ± 7% relative humidity. Four-day-old, mated adult females of *M. vitrata* were transferred in groups of four or five individuals to transparent cylindrical plastic cups (3 cm diameter × 3.5 cm height) and kept for 24 h to allow for oviposition. Ovipositing females were fed using small pieces of filter paper moistened with 10% honey solution. Cups carrying eggs were kept at the same experimental conditions until hatching by the first instar caterpillars, which were subsequently transferred to large cylindrical plastic containers (11 cm height × 16.5 cm diameter) containing sprouting cowpea grains as a feeding substrate.

Colonies of *T. javanus* were reared on *M. vitrata* first instar (three-day-old) caterpillars submitted to parasitization by *T. javanus* mated females. Parasitized caterpillars were reared on sprouting cowpea grains until pupae stage. Emerged adults were fed with a honey solution.

2.2. Reproductive Tract Morphology and Ovary Anatomy. Three-day-old adult females were dissected in a phosphate-buffered saline (PBS) solution to carefully recover the reproductive system. The specimens were prefixed in 2.5% glutaraldehyde in cacodylate buffer at 4°C during the night. Once fixed, the samples were washed (3 × 10 min) in cacodylate buffer. Postfixation was performed in 2% osmium tetroxide in the same buffer for 1 h at room temperature. Afterwards, the reproductive systems were carefully rinsed with distilled water and washed (3 × 10 min) in 50%, 70%, 90%, and 100% alcohol. Samples were subsequently placed for 1 h in a solution of EMbed 812 Resin (EMS) diluted at 50% in absolute alcohol, were rested overnight at room temperature, and were then transferred to a second, freshly prepared EMbed 812 Resin for 24 h at +60°C for polymerization. Semithin sections were then obtained using an ultra-microtome and stained with methylene blue.

We also examine the egg development within ovariole by dissecting female wasps in PBS at different time intervals after adult emergence (24, 48, 72, and 96 hours). After dissecting the ovaries, ovarioles were removed and fixed in 4% paraformaldehyde. Samples were washed for 5 minutes in PBS and stained either with DAPI (for DNA staining) or phalloidin (for actin staining) by incubation of the specimens for 30 minutes in a solution containing fluorescent phalloidin and DAPI markers diluted at 1/1000 in PBT1%, respectively. Samples were rinsed for 10 minutes in PBS and distilled water and then dried and stored at +4°C for observation of change in the contents of the ovariole using a fluorescence microscope (Zeiss Axiovert 200M equipped with Zeiss AxioCam MRm). Images were processed with ImageJ software [2020].

2.3. Ovariole Counts. To examine the effect of the *M. vitrata* host quality on ovariole number in adult female, one hundred and sixty (160) each of first instar (two-day-old), old first instar (three-day-old), and second instar (four-day-old) *M. vitrata* caterpillars, respectively, were submitted individually to parasitization by three-day-old *T. javanus* females. Caterpillars chosen were well-fed and of uniform size. Each was permitted to be stung once and then reared individually on sprouting cowpea grains in plastic cups (diameter: 9 cm on base and 12 cm on top; height: 4.5 cm) until egression of the parasitoid larva from the host and spinning of the cocoon for the pupal stage. Pupae were then collected in the plastic cups until adult emergence. Thirty-one (31) females (per host group) more than 24 h age were dissected in PBS under a stereomicroscope and the number of ovarioles per ovary was counted.

2.4. Estimation of Egg Production in Female T. javanus. Parasitoid females used in this study were mated and fed using 10% honey solution but not allowed to oviposit. Twenty (20) 12-hour-old and twelve (12) 72-hour-old females were

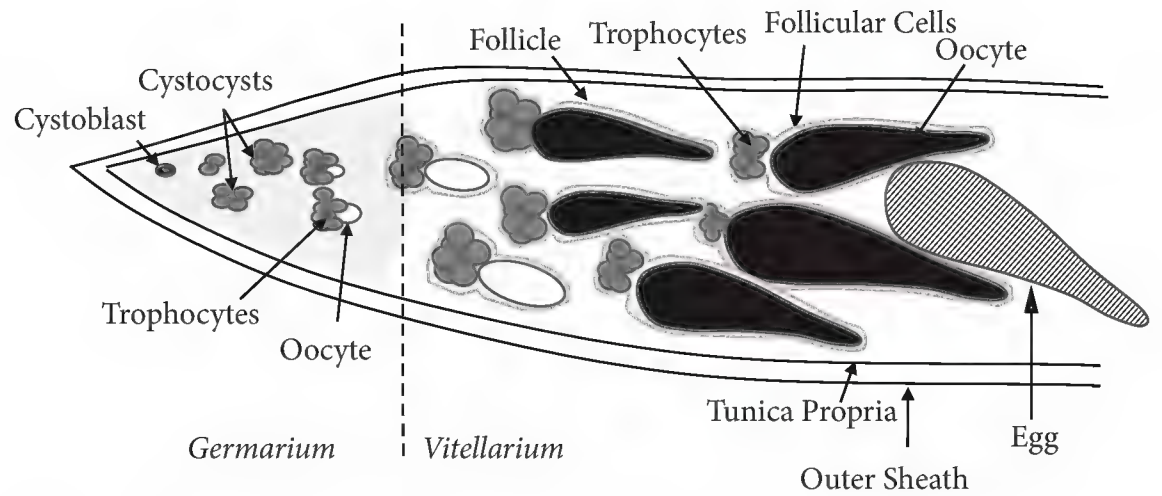

FIGURE 1: Schematic representation of the ovariole of *Therophilus javanus* showing differentiated oocyte and accompanying nurse cells (trophocytes) within the ovariole. Immature eggs recorded in our enumeration are large individual egg chambers (follicle) located in the vitellarium whose oocyte displayed an ovoid form and had a slender tapering stalk at their posterior end in solid black color, and mature eggs recorded are large individual egg chambers (egg) of ovoid form that had a slender tapering stalk at their posterior end in black striped color.

dissected in PBS and observed under a stereomicroscope. Because eggs chambers in *T. javanus* are translucent white, dissected ovaries were placed in red neutral solution for five (5) minutes to easily observe immature and mature eggs that were thus colored in red. Each ovariole was then detached and opened in PBS solution to count the number of eggs per ovariole. Large size individual eggs, still accompanied by nurse cells, were categorized as "immature eggs" (indicated by solid black color in Figure 1) and well-formed eggs that displayed an ovoid form and had a slender tapering stalk at their posterior end as "mature" eggs (black striped in Figure 1). We counted both immature eggs and mature eggs to estimate the egg production in *T. javanus*. Small size eggs chambers that were not individually differentiated were not counted (solid white color in Figure 1).

FIGURE 2: General morphology of *Therophilus javanus* female reproductive system, showing the two ovaries (Ov), the venom gland composed by two filaments (Fvg), Dufour's gland (Dg), the ovipositor (Op), and the two ovipositor sheaths (Ops). Bar 1 mm.

2.5. The Effect of Host Age at Oviposition and Female T. javanus Age after Emergence on Mature Egg Production. *Maruca vitrata* first instar (two-day-old) and second instar (four-day-old) caterpillars, well-fed and of uniform size, were submitted individually for parasitization by three-day-old *T. javanus*. Each was permitted to be stung once and then reared individually on sprouting cowpea grains in plastic cups (diameter: 9 cm on base and 12 cm on top; height: 4.5 cm) until egression of the parasitoid larva from the host and spinning of the cocoon for the pupal stage. Pupae were then collected in the plastic cups until adult emergence. Thirty (30) emerged female wasps of increasing age (one, two, three, four, and five days after adult emergence) were dissected in PBS under a stereomicroscope. Ovaries were removed and placed in red neutral solution for five minutes. The total number of mature eggs (black striped in Figure 1) in the ovariole was counted per ovary.

2.6. Data Analysis. Data were collected from February 2015 to February 2017 and general linear models with Poisson errors and log-link function, corrected for overdispersion, were used, (1) to test which host caterpillar stages impact the ovariole number in female parasitoid; (2) to test the effect of the female age on (i) the number of eggs (immature eggs + mature eggs) per ovariole, (ii) the number of eggs per ovary, and (iii) the number of eggs per female; (3) to probe the link between the number of ovarioles and the number of eggs in females; and (4) to investigate to what extent host caterpillar stage or parasitoid female age impacts the number of mature eggs per female in *T. javanus*. Multiple comparisons were carried out using the *glht* function of the "multcomp" package in the R software [20] to determine significant differences among the mean number of ovarioles per female (at the 0.05 significance level). The statistical software package R 3.3.2 [21] was used for all statistical analyses.

3. Results

3.1. General Morphology of Therophilus javanus Female Reproductive System. The *T. javanus* female reproductive system consisted of a pair of globular-shaped ovaries housing several ovarioles, a spermatheca, a Dufour's gland, a venom gland, composed of a venom duct and two venom gland filaments, and the wasp ovipositor (Figure 2).

3.2. Impact of Host Quality on the Number of Ovarioles per Female. The mean number of ovarioles per female was found to be significantly influenced by host age at the moment of oviposition (GLM: $\chi^2 = 3.6358$, df = 2, $p < 0.05$) (Figure 3). In general, the number of ovarioles varied between the three

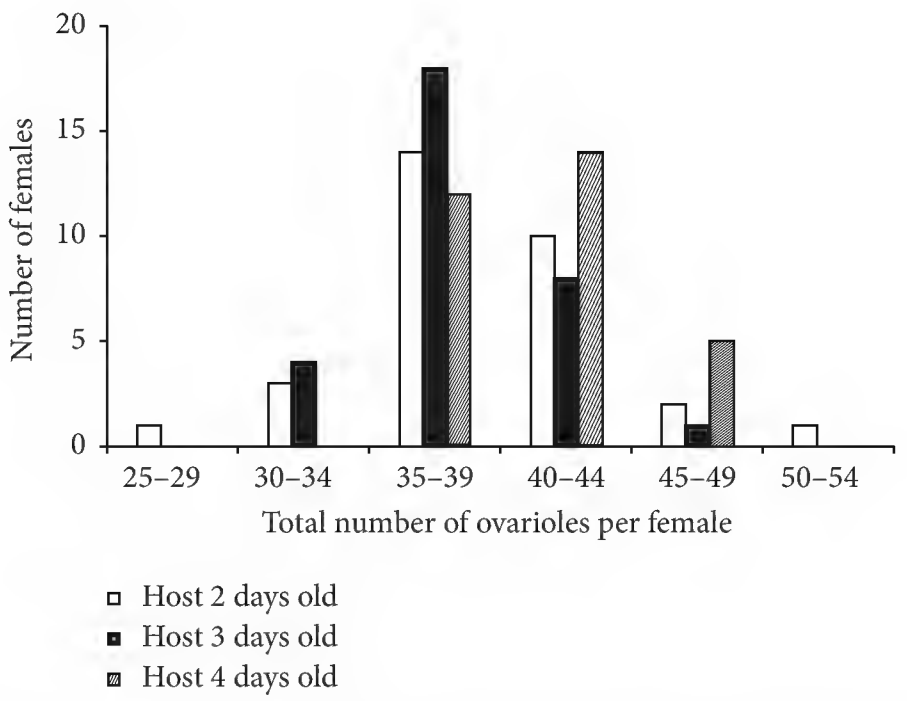

Figure 3: Variation of number of ovarioles in *Therophilus javanus* female ($n = 31$) depending on the host caterpillar age at time of parasitism (two-day-old caterpillars, three-day-old caterpillars, and four-day-old caterpillars).

females categories and was increased as the host caterpillar increased in size at the moment of oviposition. The average count in one-day-old females emerging from L1 two days old, L1 three days old, and L2 four days old is 38.36 ± 4.42 ($n = 31$); 38.16 ± 3.20 ($n = 31$); and 40.87 ± 3.15 ($n = 31$), respectively.

3.3. Egg Development within the Ovariole. *Therophilus javanus* ovarioles belong to the polytrophic meroistic type. Egg development occurred anteriorly to posteriorly along the ovariole, with two distinctly recognizable regions: the germarium and the vitellarium. The germarium contained a number of spherical cells observed as either free or clustered (Figure 4(a)). Cell nuclei size increased as they progressed along the germarium. The vitellarium is the posterior region of the ovariole, where egg chambers (follicles) are formed and grown. In *T. javanus* vitellarium, nurse cells were disposed at the top of the oocyte, all being surrounded by a sheath composed of follicular cells (Figure 4(b)). During progression of the follicles from the anterior to the posterior part of the vitellarium, vitellogenesis takes place and the size of the oocyte increases (Figure 4(c)). A cross-section of the entire ovary shows that egg chambers were in different maturation stages within and between ovarioles (Figure 4(d)). Well-differentiated oocytes (with chorion) displayed an ovoid shaped form and had a slender tapering stalk at their posterior end. Mature eggs measured $160.9 \pm 6.9\ \mu$m ($n = 20$) in length with widths ranging from $25.3 \pm 2.6\ \mu$m ($n = 20$) (anterior pole) to $9.4 \pm 1.3\ \mu$m ($n = 20$) (posterior/basal pole) (Figure 4(e)).

3.4. Impact of T. javanus Female Age on the Number of Eggs. The number of eggs (both immature and mature) per female ranged from 1 to 88 and from 349 to 476 in 12-hour-old and 72-hour-old females, respectively. The overall mean number per female increased with the female age (GLM: $\chi^2 = 6481.2$, df $= 1$, $p < 0.001$). The number of eggs ranged from 0 to 6 and from 3 to 21 per ovariole, 12 hours and 72 hours after female emergence, respectively (Table 1). As expected, the number of eggs per female was found to be significantly influenced by the total number of ovariole per female (GLM: $\chi^2 = 233.4$, df $= 1$, $p < 0.001$).

Table 1: Egg (immature + mature eggs) number (mean number ± SD) in female *Therophilus javanus* after emergence. Caterpillars were three days old at the moment of oviposition.

	Number of eggs	
	12 h after emergence	72 h after emergence
Ovariole	0.9 ± 1.2^b	11.2 ± 2.7^a
Female	30.7 ± 31.2^b	407.7 ± 45.7^a

Mean number of eggs (immature eggs + mature eggs) per ovariole and per female at 12 and 72 h after adult emergence. Eggs that were attached by nurse cells were recorded as immature eggs (black solid color in Figure 1), and well-formed eggs who displayed an ovoid form and had a slender tapering stalk at their posterior end were recorded as mature eggs (black striped color in Figure 1). Means followed by different letters between columns are significantly different between females at 12 h ($n = 20$) and 72 h ($n = 12$) after adult emergence only according to GLM with "quasi-Poisson distribution" and log-link function ($p < 0.05$).

3.5. Impact of Host and Female Wasp Age on the Egg Load. Overall, the mean number of mature eggs per female was found to be significantly influenced by host age (GLM: $\chi^2 = 44.4$, df $= 1$, $p < 0.001$) and parasitoid female age (GLM: $\chi^2 = 16600.9$, df $= 4$, $p < 0.001$). Females that emerged from four-day-old host caterpillar at oviposition had a higher mean number of mature eggs. (Figure 5).

4. Discussion

Therophilus javanus belongs to an important braconid family, the Agathidinae, whose reproductive biology is largely unknown. Our study is the first of its kind highlighting major characteristics of *T. javanus* reproductive biology and provides the basis for deploying this parasitoid as a biological control agent against the cowpea pod borer *M. vitrata* in West Africa and elsewhere.

The female reproductive tract of *T. javanus* presents a classical basic morphological organization, similar to the one described in braconids and other Agathidinae species, e.g., *Agathis pumila* (Hymenoptera: Braconidae) (Ratzeburg) [10]. However, differently from other braconids, the follicles are not organized in a string within *T. javanus* ovarioles but rather appear as "free" egg chambers. This kind of organization resembles to some extent what have been described in some Eulophidae parasitoids (e.g., *Palmistichus elaeisis* (Hymenoptera: Eulophidae)) [22].

In contrast to the insect model *Drosophila*, scarce data is available on oocyte size and number and on ovariole number in parasitoid species [23]. In some parasitoid species (e.g., ichneumonids), the number of ovarioles was shown to be a good indicator of fecundity [24]. Ovariole number is largely species-dependent but may show plasticity as a function of biological or environmental factors [25–27]. Our observations suggest that *T. javanus* displays quite a

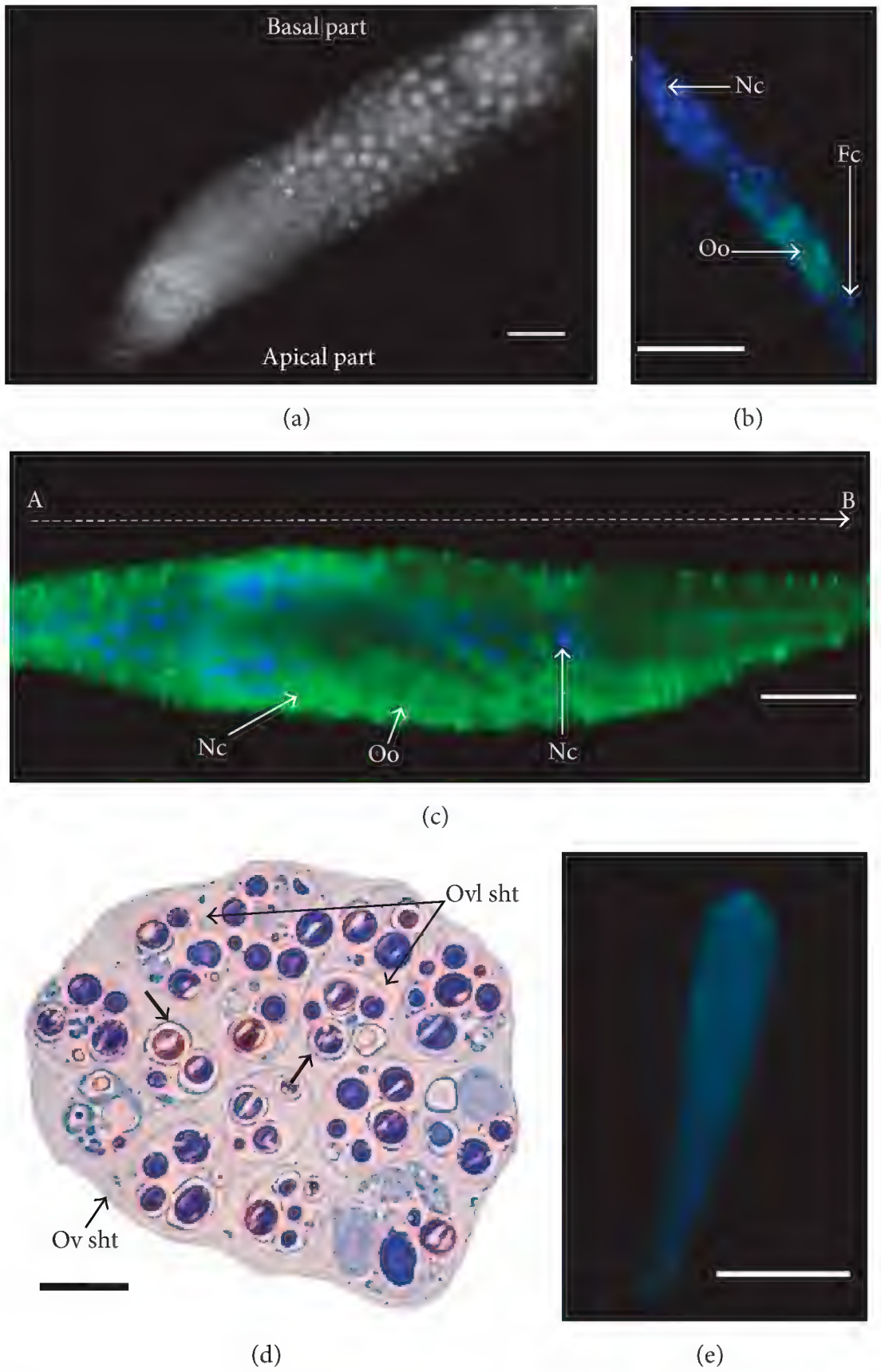

FIGURE 4: Oogenesis and ovarioles organization in *Therophilus javanus*. (a) View of the anterior region of *T. javanus* ovariole (germarium), indicating cell nuclei that increase in size along the germarium. Bar 20 μm. (b) Individual follicle taken out from a *T. javanus* ovariole. The picture shows the disposition of the nurse cells (Nc) at the top of the oocyte (Oo). The nuclei of follicular cells (Fc) from the sheath surrounding the follicle can also be observed. Bar 50 μm. (c) Basal part of the ovariole of *T. javanus*. Follicles are in increasing development stages along the vitellarium (from A to B). On the left of the picture, follicles display small oocytes and trophocytes with a large nucleus. On the right, oocytes have increased in size thanks to progression of vitellogenesis. Note the network of actin fibers (in green) surrounding the egg chamber. Nurse cells (Nc); oocyte (Oo). Bar 50 μm. (d) Organization of the ovarioles and follicles within *T. javanus* ovaries: cross-sections of an ovary containing 15 ovarioles. The ovary is enveloped by the ovarian epithelial sheath (Ov sht) and each ovariole is surrounded by an epithelial sheath (Ovl sht). In the section, oocytes are in different stages within and between ovarioles. Some eggs with a chorion can be observed (shown by arrows). Semithin section stained with methylene blue. Bar 50 μm. (e) The mature egg of *T. javanus*. The egg has an ovoid shape and a slender tapering stalk at its posterior end. Bar 50 μm.

variable number of ovarioles, which has also been commonly reported in noncyclostome Braconidae subfamilies, including in Agathidinae (from 4 to 30) [6, 28]. In *T. javanus*, the highest number of ovarioles has been observed in females issued from larger hosts (i.e., second instar *M. vitrata* larvae). Impact of host instar on parasitoids ecological and biological traits has been reported in several studies [29–32]. For example, the average number of eggs in *Microplitis rufiventris* Kok (Hymenoptera: Braconidae) was higher in females that emerged from *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) parasitized at younger larval stage [33].

Our study has demonstrated that, in *T. javanus*, egg load is influenced by females' age. The largest average number of mature eggs (177.97 ± 2.62) was counted in five-day-old females that emerged from larger hosts (L2, four-day-old). This confirms observations in other Braconidae that larger

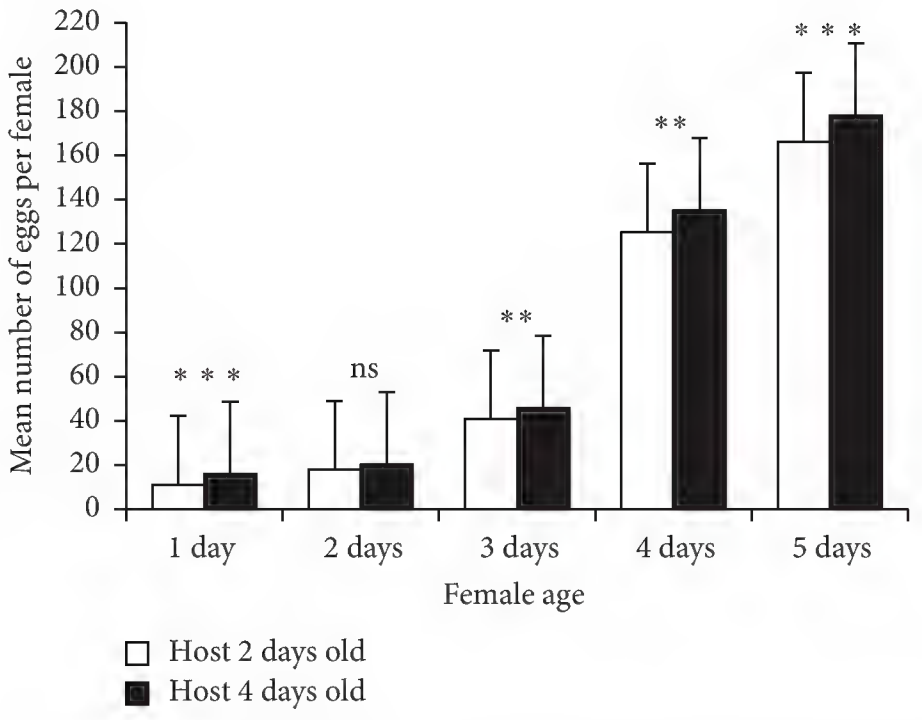

FIGURE 5: Effect of host age (at oviposition) and female age on the mature egg (individual egg chambers located in the vitellarium who displayed an ovoid form and had a slender tapering stalk at their posterior end (in black striped color in Figure 1)) load in *Therophilus javanus*. Caterpillars were two and four days old at the moment of oviposition. Error bars represent the standard errors of the means ($n = 30$). Means that were significantly different between two- and four-day-old hosts only according to GLM and Tukey HSD test are indicated by asterisks ($^{**}p < 0.01$; $^{***}p < 0.001$; ns: nonsignificant).

parasitoids are issued from larger host larvae and larger females lay higher number of eggs [34]. In spite of the uniform size of *M. vitrata* caterpillars used for parasitization, we did observe a variable number of eggs in *T. javanus* females, which has been demonstrated to depend on abiotic or biotic factors in the Agathidinae *B. vulgaris* and *A. gibbosa* [9, 11]. For instance, the number of eggs laid by *B. vulgaris* varies depending on the size of the wasp female and on temperature at adult emergence, whereas mating is known to decrease egg load in *A. gibbosa*. In three-day-old mated *T. javanus* females, the mean number of mature eggs was much smaller than the number of immature ones (50 and 357, resp.), suggesting a large potential fecundity. As generally observed in koinobiont species including some Agathidinae [35], this could be related to the ability of *T. javanus* females to continue oogenesis after emergence, provided there are sufficient protein sources to maintain oogenesis and complete egg maturation. In fact, the impact of adult life time protein deficiency on oogenesis has been previously documented in *Microterys flavus* (Hymenoptera: Encyrtidae) [36]. Like for *A. pumila* [10], *T. javanus* females do not host-feed and may probably need to take additional protein from nonhost food sources after emergence, possibly slowing down egg maturation.

As described for other koinobiont species that attack hosts at early stages [35], *T. javanus* displays very tiny eggs (0.1 mm in length and 0.025 mm for its larger width). This minute size and the tear shape of *T. javanus* eggs correspond to previous descriptions of Agathidinae eggs such as those produced by *B. vulgaris* (Cress.), *A. pumila,* and *A. gibbosa* [9–11]. *T. javanus* females are synovigenic; that is, they emerge with high numbers of immature eggs and only few mature eggs but continue to produce eggs throughout the adult stage, implying that females can start to oviposit in host caterpillars just after emergence. This ability to start oviposition just after emergence has been mentioned in other Agathidinae species, that is, *A. gibbosa*, *A. pumila,* and *B. vulgaris* [9–11]. In contrast to what was observed in the ovarioles of *A. pumila* and most Braconidae, however, both developing and mature eggs were found at the same level in the basal part of *T. javanus* ovariole. This suggests that *T. javanus* females might have developed a mechanism allowing them to lay only mature eggs in the host.

The current mass-rearing protocol, using three-day-old *T. javanus* females, stems from a desire to maximize the production of mated females as recommended for parasitoid rearing in biological control programs [37, 38]. Our findings, however, show that egg production was relatively low (40.8 ± 8.6) during the first three days following adult emergence, suggesting that better outputs could be obtained using females older than three days. Our results also show that egg production in *T. javanus* is influenced by the size or instar of the caterpillar host. From a mass-rearing perspective, this suggests that the overall fecundity of *T. javanus* could be improved by selecting second instar caterpillars. However, under field conditions, the fecundity of foraging *T. javanus* females could be influenced by the size of the available *M. vitrata* life stages. Also, along with preliminary observations that *T. javanus* females did not perform host feeding on *M. vitrata* caterpillars, it will be important to investigate how feeding on sugar sources may impact the fecundity of *T. javanus* females. In fact, synovigenic parasitoids that do not feed on host are usually able to use sugar foods for oogenesis [18]. Notably, cowpea itself, the major crop hosting caterpillars of *M. vitrata*, secretes extrafloral nectar [39], which may provide an adequate source of sugar food for foraging female parasitoids. We expect similar extrafloral nectar to be present on other important, wild-occurring host plants such as *Sesbania rostrata* (Fabales: Fabaceae) and *Tephrosia platycarpa* (Fabales: Fabaceae), known to harbor important pod borer populations [40], which might also be visited by foraging *T. javanus* females.

5. Conclusion

Biological, rather than pesticidal, control of *M. vitrata* offers numerous advantages, especially in poor rural areas where the cost of pesticides, along with human and environmental exposures, becomes unsustainable or prohibitive. Even with pesticides, however, difficulty in recognizing the presence of *M. vitrata* prior to destructive crop predation makes conventional crop protection methods challenging. Our findings provide the first baseline information toward elucidating several factors influencing the reproductive biology in *T. javanus*, a promising biological control candidate against *M. vitrata* in West Africa. The fact that *T. javanus*, along with its high level of potential fecundity, may also demonstrate a greater facility for identifying and taking advantage of the presence of *M. vitrata* potentially enhances its use as a biological control to a great degree, while also affording the many cost, human, and environmental advantages of not using chemical pesticides.

Conflicts of Interest

All authors declare no conflicts of interest.

Acknowledgments

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